GALACTURONOSYLTRANSFERASES, NUCLEIC ACIDS ENCODING SAME AND USES THEREFOR

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims benefit of United States Provisional Patent Application No. 60/445,539 filed February 6, 2003, which is incorporated in its entirety herein by reference to the extent not inconsistent herewith.

BACKGROUND

This invention relates to plant physiology, growth, development, defense and, in particular, to plant genes, termed galacturonosyltransferases (GALATs), nucleic acids encoding same and the uses therefor.

Pectins are the most complex polysaccharides in the plant cell wall. They comprise 30-40% of the primary wall of dicots and non-graminaceous monocots, and ~ 10% of the primary wall in the grass family. Pectins are a family of polysaccharides[6,8,27] that include homogalacturonan (HGA) (Fig. 1), rhamnogalacturonan-I (RG-I) (Fig. 2) and rhamnogalacturonan II (RG-II) (Fig. 3) as well as xylogalacturonans (XGA)[32,34,38]and apiogalacturonans.[6,37] While the specific structure of each of these polysaccharides differs as shown in Figs. 1-3, they are grouped into one family since they appear to be linked to each other in the wall and they each contain α-D-galacturonic acid connected by a 1,4-linkage.

HGA is the most abundant pectic polysaccharide, accounting for ~55%-70% of pectin[39]. HGA is a linear homopolymer of α1,4-linked D-galactosyluronic acid that is partially methylesterified at the C6 carboxyl group and may be partially acetylated at O-2 and/or O-3[8] (Fig. 1). Some plants also contain HGA that is substituted at the 2 or 3 position by D-apiofuranose, the so-called apiogalacturonans (AGA)[36,37] and/or HGA that is substituted at the 3 position with D-xylose[32-35], so-called xylogalacturonan (XGA). RG-II is a complex polysaccharide

that accounts for approximately 10-11% of pectin[8,39]. RG-II has an HGA backbone with four structurally complex side chains attached to C-2 and/or C-3 of the GalA[8,27] (Fig. 3). Rhamnogalacturonan I (RG-I) accounts for 20-35% of pectin[39] (Fig. 2). RG-I is a family of polysaccharides with an alternating [→4)-α-D-GalA-(1→2)- α-L-Rha-(1→] backbone in which roughly 20-80% of the rhamnoses are substituted by arabinan, galactan, or arabinogalactan side branches[6,8,30].

Pectins are believed to have multiple roles during plant growth, development, and in plant defense responses. For example, pectic polysaccharides play essential roles in cell wall structure[43], cell adhesion[44] and cell signaling[45,46]. Pectins also appear to mediate pollen tube growth[47] and to have roles during seed hydration[48,49], leaf abscission[50], water movement[51], and fruit development[47,8]. Oligosaccharides cleaved from pectin also serve as signals to induce plant defense responses[52,53]. Studies of mutant plants with altered wall pectin reveal that modifications of pectin structure leads to dwarfed plants[43], brittle leaves[44], reduced numbers of side shoots and flowers[54], malformed stomata[44] and reduced cell adhesion[55].

Although pectins appear to have multiple roles in plants, in no case has their specific mechanism of action been determined. One way to directly test the biological roles of pectins, and to study their mechanisms of action, is to produce plants with specific alterations in pectin structure. This can be done by knocking out genes that encode the pectin biosynthetic enzymes. Such enzymes include the nucleotide-sugar biosynthetic enzymes and the glycosyltransferases that synthesize the pectic polysaccharides. Each glycosyltransferase is expected to transfer a unique glycosyl residue in a specific linkage onto a specific polymeric/oligomeric acceptor. To date, only five[56-59,136] of the more than 200 predicted wall biosynthetic glycosyltransferases have been funtionally identified at the gene level (i.e. enzyme activity of the gene product proven), and none of these have been shown to encode pectin biosynthetic enzymes.

Based on the known structure of pectin, at least 58 distinct glycosyl-, methyl- and acetyl-transferases are believed to be required to synthesize the family of

polymers known as pectin. As shown in the review by Mohnen, D. (2002) "Pectins and their Manipulation", G.B. Seymour *et al.*, Blackwell Publishing and CRC Press, Oxford, England, pp. 52-98, and Table I below, a minimum of 4-9 galacturonosyltranferases are predicted to be required for the synthesis of HGA, RG-I, RG-II and possibly for the synthesis of the modified forms of HGA known as XGA and AGA. The present invention relates to the identification of the first gene, *GALAT1,* encoding a galacturonosyltranferase and related genes thereto. The studies disclosed hereinbelow led the inventors to conclude that the gene *GALAT1* encodes the enzyme known as *UDP-GalA:Homogalacturonan α-1,4-Galacturonosyltransferase.*

Table I. *List of galacturonosyltransferase activities predicted to be required for pectin biosynthesis*[9]

Type of GalAT	Working[1] Number	Parent polymer[2]	Enzyme[3] Acceptor substrate *Enzyme activity*	Ref for Structure
D-GalAT	1	HGA	*GalAα1→4GalA *α1,4-GalAT*	[27]
D-GalAT	2	RG-I	L-Rhaα1→4GalA *α1,2-GalAT*	[27-29]
D-GalAT	3	RG-II	L-Rhaβ1→3Api*f* *α1,2-GalAT*	[30,31]
D-GalAT	4	RG-II	L-Rhaβ1→3Api*f* *β1,3GalAT*	[30,31]
D-GalAT	5 ?[4]	RG-I/HGA	GalAα1→2LRha *α1,4-GalAT*	---
D-GalAT	6 ?	RG-II/HGA	GalAα1→4GalA *α1,4-GalAT*	---
D-GalAT	7?	XGA	GalAα1→4(Xyl β1→3)GalA[5] *α1,4-GalAT*	[32-35]
D-GalAT	8 ?	AGA	GalAα1→4(Api*f* β1→2)GalA *α1,4-GalAT*	[36,37]
D-GalAT	9 ?	AGA	GalAα1→4(Api*f* β1→3)GalA *α1,4-GalAT*	[36,37]

[1]Numbers for different members of the same groups are given based on pectin structure and on the assumption that HGA is synthesized first, followed by RG-I and RG-II. The numbers were given[9] to facilitate a comparison of the enzymes, but final numbering will likely correspond to the order in which the genes are identified.
[2]HGA: homogalacturonan; RG-I: Rhamnogalacturonan I; RG-II: Rhamnogalacturonan II; XGA: Xylogalacturonan; AGA; Apiogalacturonan.
[3]All sugars are D sugars and have pyranose rings unless otherwise indicated. Glycosyltranferases add to the glycosyl residue on the left* of the indicated acceptor.
[4]The ? means the designated GalAT may be required if a different GalAT in the list does not perform the designated function.
[5]Glycosyl residue in the parenthesis is branched off the first GalA.

Over the years, membrane-bound α1-4galacturonosyltransferase (GalAT) activity has been identified and partially characterized in mung bean[10,11], tomato[12], turnip[12], sycamore[13], tobacco suspension[2], radish roots[5], enriched Golgi from pea[7],

Azuki bean[14], *Petunia*[15], and *Arabidopsis* (see Table II). The pea GalAT was found to be localized to the Golgi[7] with its catalytic site facing the lumenal side of the Golgi[7]. These results provided the first direct enzymatic evidence that the synthesis of HGA occurs in the Golgi. In *in vitro* reactions, GalAT adds [^{14}C]GalA from UDP-[^{14}C]GalA[1,60] onto endogenous acceptors in microsomal membrane preparations to produce radiolabeled products of large molecular mass (i.e. ~105 kd in tobacco microsomal membranes[2] and ≥ 500 kd in pea Golgi[7]). The cleavage of up to 89% of the radiolabeled product into GalA, digalacturonic acid (diGalA) and trigalacturonic acid (triGalA) following exhaustive hydrolysis with a purified endopolygalacturonase confirmed that the product synthesized by tobacco GalAT was largely HGA. Thus, the crude enzyme catalyzes the reaction *in vitro*: UDP-GalAT + HGA(n) → HGA(n+1) + UDP. The product produced *in vitro* in tobacco microsomes was ~ 50% esterified[2] while the product produce in pea Golgi did not appear to be heavily esterified[7]. These results suggest that the degree of methyl esterification of newly synthesized HGA may be species specific and that methylesterification occurs after the synthesis of at least a short stretch of HGA. GalAT in detergent-permeabilized microsomes from azuki bean seedlings added [^{14}C]GalA from UDP-[^{14}C]GalA onto acid-soluble polygalacturonate (PGA) exogenous acceptors[14]. Treatment of the radiolabeled product with a purified fungal endopolygalacturonase yielded GalA and diGalA, confirming that the activity identified was a GalAT comparable to that studied in tobacco and pea. The azuki bean enzyme had a surprisingly high specific activity of 1300-2000 pmol mg^{-1} min^{-1}, especially considering the large amount (3.1-4.1 nmol mg^{-1} min^{-1}) of polygalacturonase activity that was also present in the microsomal preparations. As with the product made by tobacco, no evidence for the processive transfer of galactosyluronic acid residues onto the acceptor was obtained (see below).

Table II. *Comparison of apparent catalytic constants and pH optimum of HGA-α1,4-galacturonosyltransferases*[1,2]

Enzyme[2]	Plant Source	Apparent K_m for UDP-GalA (μM)	pH optimum	Vmax (pmol $mg^{-1}min^{-1}$)	Ref
GalAT[1]	mung bean	1.7	6.0	~4700	10
GalAT	mung bean	n.d.	n.d.	n.d.	61
GalAT	pea	n.d.[5]	6.0	n.d.	62
GalAT	pea	n.d.	n.d.	n.d.	7
GalAT	sycamore	770	n.d.	?	13
GalAT	tobacco	8.9	7.8	150	2
GalAT (sol)[3]	tobacco	37	6.3-7.8	290	3
GalAT (sol)[3]	Petunia	170	7.0	480	15
GalAT (per)[4]	Azuki bean	140	6.8-7.8	2700	14

[1]Adapted from ref 6.
[2] Unless indicated, all enzymes are measured in particulate preparations.
[3] (sol): detergent-solubilized enzyme.
[4] (per): detergent-permeabilized enzyme.
[5] n.d.: not determined.

GalAT can be solubilized from membranes with detergent[3]. Solubilized GalAT adds GalA onto the non-reducing end[4] of exogenous HGA (oligogalacturonide; OGA) acceptors of a degree of polymerization of at least ten[2]. The bulk of the HGA elongated *in vitro* by solubilized GalAT from tobacco membranes[3], or detergent-permeabilized Golgi from pea[7], at roughly equimolar UDP-GalA:acceptor concentrations is elongated by a single GalA residue. These results suggest that solubilized GalAT *in vitro* acts nonprocessively, (i.e. distributively). The apparent lack of *in vitro* processivity of GalAT was recently confirmed by Akita *et al.* who, using pyridylaminated oligogalacturonates as substrates and high concentrations of UDP-GalA, showed that although OGAs can be elongated in a "successive" fashion with up to 10 GalA residues by solubilized enzyme from petunia pollen[15], the kinetics of this response suggest a distributive mode of action. We have two working hypotheses as to why GalAT *in vitro* does not appear to act processively. One hypothesis is that the solubilized enzyme or the enzyme in particulate preparations does not have the required factors, or is not present in the required complex, to act processively. An alternative hypothesis is that for a Golgi-localized enzyme that synthesizes a complex polymer in a confined

internal cellular compartment, such as GalAT, with sufficiently high concentrations of substrate, it would not necessarily be advantageous for the enzyme to act processively. In fact, the reaction velocity could be hindered under such conditions if the enzyme were processive[65].

The apparent kinetic constants and pH optimum for the characterized GalATs are shown in Table II. We have performed additional kinetic studies in tobacco and radish that suggest that solubilized and membrane bound GalAT may have unusual apparent biphasic kinetics. We tested Vo for radish GalAT at 2 μM to 80 mM UDP-GalA and obtained a biphasic curve (Fig. 4), suggesting that the kinetics of GalAT, at least in the membrane and soluble fractions, are complex. Comparable results were also obtained for the solubilized radish and tobacco enzyme. The initial Vo vs [UDP-GalA] curve was hyperbolic and appeared to reach an initial maximum Vo of ~ 300 pmol mg^{-1} min^{-1} at ~1 mM UDP-GalA, confirming previous results reported for tobacco[2,3]. However, at ≥ 2 mM UDP-GalA there was a second hyperbolic increase in GalAT activity that reached a maximum of ~2-4 nmol min^{-1} mg^{-1} with ~20 mM UDP-GalA. In crude enzyme preparations it was not possible to determine the basis for the unusual kinetics. One possibility is that two GalATs were present, one with a low Km and one with a high Km. Another possibility is that UDP-GalA is both a substrate and an allosteric regulator of GalAT. Alternatively, a more "trivial" explanation is that at low substrate concentrations the kinetics of GalAT were effected by a catabolic enzyme (e.g. a phosphodiesterase) in the enzyme preparation.

As a first step towards elucidating the role of galacturonosyltransferase (GALAT) in pectin synthesis, the inventors herein identified an *Arabidopsis* gene encoding alpha1,4- galacturonosyltransferase 1 (GALAT1). The database searches using the amino acid sequence of the GALAT1 identified fourteen additional *GALAT* family members and ten *GALAT*-like genes. The identification of these genes and the availability of the sequence information allow the characterization of the enzyme, the use of these genes to produce mutated enzymes *in vivo* and *in vitro*, and transgenic plants producing modified pectins, and

studies of the role of a specific GalAT in pectin synthesis. The advantages of the present invention will become apparent in the following description.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid molecule encoding the polypeptide having galacturonosyltransferase (GalAT) activity. The *GALAT 1* disclosed herein represents the first functionally proven pectin biosynthetic glycosyltransferase gene isolated from plants. Also provided are additional 14 *GALAT* gene family members and 10 *GALAT*-like genes predicted to have galacturonosyltransferase activity. The identification and availability of the nucleic acid molecules as a member of the *GALAT* gene superfamily offer new opportunities to modulate pectin synthesis *in vivo* and *in vitro* by modulating the *GALAT* gene using various art-known recombinant DNA technology. For example, transgenic plants that produce modified pectins of desired properties can be generated by manipulating the gene encoding the GALAT protein i.e., mutating the gene including coding and non-coding sequences, silencing the gene by RNAi approach, or by administering a composition that would affect the GalAT activity in the plant. Since modified pectins are predicted to affect plant growth, development, and plant defense responses, the transgenic plants thus modified are expected to have improved agricultural value. The modified pectins can be isolated from such transgenic plants according to the art-known methods and serve as gelling and stabilizing agents of improved properties in the food, neutraceutical, and pharmaceutical industries.

The inventors herein identified the first gene, *GALAT1,* which encodes a pectin biosynthetic enzyme by employing a partial purification-tandem mass spectrometry approach combined with a search of the *Arabidopsis* gene/protein database. Two genes, designated JS33 and JS36 herein, were identified as present only in the GalAT-containing fractions. As demonstrated hereinbelow, the expressed protein from the nucleic acid sequence of JS36 indeed exhibits the predicted GalAT enzymatic activity.

A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence revealed that *GALAT*1 is a member of a 15 member *GALAT* gene family in *Arabidopsis*. The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. The database search using the GALAT1 sequence further identified 10 *GALAT*-like genes as shown in Table IV. The genes disclosed herein, fifteen *GALAT* genes and ten *GALAT-like* genes thus represent the *GALAT* gene superfamily members.

The availability of the amino acid and nucleotide sequences of the *GALAT* gene superfamily members makes it possible to identify other *GALAT* homologs in other plants. The nucleotide and amino acid sequences of the *GALAT* genes can also be used to generate specific antibodies for the protein.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the trimeric region of homogalacturonan (HGA). HGA is a linear homopolymer of alpha-1,4-linked galacturonic acid that may be methylesterified at C6 and acetylated at O2 or O3. Substituted galacturonans, such as RG-II and apiogalacturonan, have an HGA backbone.

Fig. 2 shows the representative structure of rhamnogalacturonan I (RG-I). RG-I has an alternating [→4)-alpha-D-GalpA-(1→2)-alpha-L-Rhap-(1→) backbone in which roughly 20-80% of the rhamnoses are substituted by arabinans, galactans, or arabinogalactans.

Fig. 3 shows the representative structure for rhamnogalacturonan II (RG-II). RG-II has a backbone of 1,4-linked alpha-D-GalpA residues. GalA residues are also present in RG-II side chain A.

Fig. 4 illustrates the GalAT kinetics in radish microsomal membranes. Radish microsomal membranes (60-80 μg protein) were incubated with 70 μg of OGA (DP 7-23) and the indicated concentrations of UDP-GalA. Each reaction

contained a small concentration of UDP-[^{14}C]GalA (2-3.6 μM) with larger amounts of nonradioactive UDP-GalA. The precipitated reaction products were measured by liquid scintillation counting. The data are the averages of duplicate samples from three separate experiments. The Y axis is specific activity (pmole $min^{-1}mg^{-1}$).

Fig. 5 shows the outline of the strategy to identify the gene for GalAT. The sequenced *Arabidopsis* genome allowed the use of a function-based partial purification-mass spectrometry approach to identify the putative galacturonosyltransferase genes. The sample analyzed in each lane is as follows: lane 1: homogenate, lane 2: total membranes, lane 3: solubilized proteins, lane 4: initial anion exchange purification step.

Figs. 6A and 6B show the results of RT-PCR experiments; 6A shows the results of JS33, JS36, and JS36L (a GalAT family gene with 63% identity to JS36) using *Arabidopsis* flower (F), root (R), stem (S), and leaf (L) RNA, and B shows the RT-PCR control using *Arabidopsis* actin gene in the same tissues.

Fig. 7 is a schematic representation of the transmembrane spanning region and the conserved amino acids in the *Arabidopsis thaliana GALAT* gene family. The relative position of the strictly conserved residues among the members of the proposed *GALAT* family is numbered as for JS36 (i.e., GALAT1). The striped region from residues 22-44 represents the predicted transmembrane region.

Fig. 8 demonstrates that recombinant JS36 (At3g61130) has galacturonosyltransferase (GalAT) activity. Human embryonic kidney cells (HEK293) were transiently transfected with the pEAK vector alone, or with pEAK vector containing the truncated versions of JS33 or JS36. Total media (1); protein immunoabsorbed from the medium using anti-HA epitope:Protein A Sepharose (2); and protein immunoabsorbed from the medium using anti-HA epitope:Protein G Sepharose (3) were tested for GalAT activity. Data are the average [^{14}C]GalA incorporated into product from duplicate reactions from three separate experiments.

Fig. 9 shows the relationship of the *Arabidopsis* GalAT superfamily including the GalAT family and the GalAT-like family. The Neighbor-Joining Tree is based on a sequence alignment generated by ClustalX.

DETAILED DESCRIPTION OF THE INVENTION

In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

In the present application, the designation, "*GALAT*", is used to denote the gene for galacturonosyltransferase, "GALAT" is used to denote the protein encoded by the gene, and "GalAT" is used to indicate galacturonosyltransferase enzyme activity.

The term, "polypeptide", is used herein interchangeably with "protein" to indicate a product encoded by a given nucleic acid.

The terms, "identity" or "similarity" as used herein, are intended to indicate the degree of homology between the two or more nucleic acid or amino acid sequences. The degree of identity or similarity can be determined using any one of the computer programs that are well known in the art. The National Center for Biotechnology Information (NCBI) website on the internet provides detailed description and references necessary for this subject. Also see Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* **90**:5873-5877; Altschul *et al.* (1997) *Nucl. Acids. Res.* **25**:3389-3402. In the present application, the percent amino acid identity and similarity among the *GALAT* gene family and *GALAT*-like gene family members were carried out using the NCBI Pairwise Blast and Matrix Blosum62 using the GALAT1(JS 36) amino acid sequence.

A "corresponding" nucleic acid or amino acid or sequence of either, as used herein, is one present at a site in a GALAT molecule or fragment thereof that has the same structure and/or function at a site in another GALAT molecule, although the nucleic acid or amino acid position may not be identical.

The term "gene" is used herein in the broadest context and includes a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or nontranslated sequences (i.e., introns, 5'- and 3'-untranslated sequences), or mRNA or cDNA corresponding to the coding regions (i.e., exons) and 5'- and 3'-untranslated sequences.

The meaning of a "homolog" as used herein is intended to indicate any gene or gene product which has a structural or functional similarity to the gene or gene product in point. For example, a new homolog of a given *GALAT* gene can be identified either by a database search using the amino acid or nucleic acid sequences of a given *GALAT* gene or by screening appropriate cDNA or genomic libraries according to the art-known methods.

An "expression vector" as used herein, generally refers to a nucleic acid molecule which is capable of expressing a protein or a nucleic acid molecule of interest in a host cell. Typically, such vectors comprise a promoter sequence (e.g. TATA box, CATTbox, enhancer etc) fused to a heterologous sequence (i.e., a nucleic acid of interest), sense or antisense strand, followed by a transcriptional termination sequence, a selectable marker, and other regulatory sequences necessary for transcription and translation of the nucleic acid of interest. A plant expressible promoter is a promoter comprising all the necessary so called regulatory sequences for transcription and translation of a gene of interest in plants. The linkage between the heterologous sequence and the regulatory sequences (e.g., promoter) is "in operable linkage" when a desired product can be made from the heterologous sequence under the control of the given regulatory sequences. An "expression vector" is often used interchangeably with an "expression construct" in this sense.

The term "transgenic plant" as used herein refers to a plant that has been transformed to contain a heterologous nucleic acid, i.e., a plant expression vector or construct for a desired phenotype. The transgenic plant is intended to include whole plant, plants parts (stems, roots, leaves etc.) or organs, plant cells, seeds, and progeny of same. The transgenic plant having modified pectin of the present application is one that has been generated by manipulating the gene encoding the GALAT protein. This can be achieved, for example, by mutating the gene, silencing

the gene by RNAi approach, or by knocking out the gene. The transgenic plants of the invention are predicted to have properties such as changes in organ and plant size, water transport properties, ease of removal of leaves and fruits via effects on abscision, pollen development and release, fruit ripening, root mucilage production, root growth, root cell cap production and separation, stem elongation, shoot growth, flower formation, tuber yield, defense responses against pathogens, and stomata opening[8]. Thus, the invention provides new means of improving plants of agricultural value. The "modified" pectins are those that exhibit structures and properties (e.g., gelling and stabilizing) different from those of the pectins naturally present in plants. Since galacturonic acid is a component of each of the pectic polysaccharides (i.e. HGA, RG-I, RG-II and XGA), a modification of the GalATs that add the specific GalAs into the specific polysaccharides is expected to modify the unique polymers. Such changes in pectin structure would affect multiple pectin properties including ionic interactions between HGA regions, gelation properties, dimer formation of RG-II molecules, length and degree of branching of RG-I, and side branch structure of RG-II. Such modifications are predicted to not only affect the biological function of pectin in plants, and the chemical and biological properties of pectin extracted and used by the food and cosmetic industries, but also properties that affect the use of pectin as a biopolymer for industrial processes, as a drug delivery polymer, and pectins of medicinal and neutraceutical properties in human and animal health.

The term "mutation" as used herein refers to a modification of the natural nucleotide sequence of a nucleic acid molecule made by deleting, substituting, or adding a nucleotide(s) in such a way that the protein encoded by the modified nucleic acid is altered structurally and functionally. The mutation in this sense includes those modifications of a given gene outside of the coding region.

The present invention provides polypeptides and nucleic acids encoding the polypeptides belonging to a family of the pectin biosynthetic enzyme, galacturonosyltransferase (GALAT). Pectins have been implicated in a broad range of plant growth phenomena including pollen tube growth[47], seed hydration[48-49], leaf abscission[50], water movement[128], and fruit development[8]. In addition, pectic oligosaccharides serve as signals[45] during plant development[45] and induce plant

defense responses[52-53]. Mutant studies have shown that altered pectin structure leads to dwarfed plants[43], brittle leaves[44], reduced numbers of side shoots and flowers[129], and plants with reduced cell-cell adhesion[130, 55]. Therefore, the present invention provides the molecular and biochemical tools needed to identify additional glycosyltransferases involved in branching of the backbones, and would allow the generation of plants with altered pectin structure. While the 25 genes disclosed herein represent only ~0.1% of the ~28,000 genes in *Arabidopsis*, they are some of the most difficult genes to identify and characterize because of a lack of commercially available acceptor substrates and activated glycosyl donor substrates.

The *GALAT1* gene has high sequence similarity to proteins expressed in other plants, thus using the sequences disclosed herein, a person of ordinary skill in the art can identify other pectin biosynthetic genes (i.e. homologs) in other plant species, including agriculturally important plants. Since pectin of very similar structure is present in the walls of all flowering plants and gymnosperms, the identification of functional pectin biosynthetic genes will greatly facilitate the engineering of plants with modified pectin and with altered growth characteristics, some of which are expected to yield plants of increased agronomical value. In addition, mutant plants with defined changes in pectin synthesis can allow the dissection of the biological role of each pectic component in plants. The pectin biosynthetic genes provide valuable tools for understanding mechanistically how pectin is synthesized. The glycosyltransferase-specific antibodies that can be generated using the sequences disclosed herein are also within the scope of the invention and allow the process of pectin assembly in the Golgi to be elucidated. A complete understanding of such a polysaccharide cellular trafficking process is unknown in any biological system.

Pectin is found in fruits and vegetables and is used as a gelling and stabilizing agent in the food industry. Pectin has been shown to have multiple beneficial effects on mammalian systems and on human health including the inhibition of cancer growth and metastasis, inhibition of cancer metastasis by binding of pectic oligosaccharides to cell surface receptors of cancer cells (US5834442, US5895784), immunomodulatory effects and stimulation of tumor

necrosis factor by macrophages (EP03983113), interaction with mucous cell lining of the duodenum and the prevention of ulcers (US4698229, US6024959); and anti-complementary activity[125]. Many cancer cells have specific carbohydrate-binding protein molecules on their cell surfaces called galectins (galactoside-binding lectins). Galectins aid in cellular interactions by binding to beta-galactose linked molecules on neighboring cancer cells. Galectin-3 is a multifunctional lectin that is involved in tumor cell adhesion, metastasis and cancer progression. Blocking galectin-3 expression in malignant human breast, papillary and tongue carcinoma cells led to reversion of the transformed phenotype and suppression of tumor growth in nude mice [117-119]. A pH-modified citrus pectin is suggested to block binding of galectins and inhibit tumor cells adhesion. Pienta *et al.* [127] showed that feeding of pH-modified pectin to rats caused a reduction in metastasis of prostate cancer. Similarly, oral administration of pectin to mice carrying colon tumors, reduced tumor size compared to control animals[114], reduced metastatic colonization of B16-F1 melanoma in the lung[120-121] and reduced human breast and colon carcinoma growth, angiogenesis, and metastasis[125]. When prostate cancer patients were fed pH-modified citrus pectin, a 30% lengthening in prostate specific antigen (PSA) doubling time was observed in 57% of the patients[122]. As progression of prostate cancer is evaluated based on the time that it takes for the PSA to double, the above observations suggested that pectins may reduce tumor size. It has also been shown that fruit-derived pectins inhibit the interaction of fibroblast growth factor 1 (FGF1) to its receptor (FGFR1)[123]. Defects in the FGF signal transduction system are known to disturb cellular regulatory processes resulting in cancer, cardiovascular disease and diabetes mellitus. The availability of the gene(s) encoding galacturonosyltransferase allows the modification of neutraceutical or pharmaceutical pectins to provide pectins with novel cell and molecule binding activities and thus, with novel and specified anticancer and other physiological activities.

In order to identify a gene(s) involved in pectin biosynthesis, the inventors used a partial purification-tandem mass spectrometry approach to identify putative *GALAT* genes from *Arabidopsis* (see Fig. 5 for strategy). GalAT from *Arabidopsis* was partially purified from detergent-solubilized enzyme by sequential passage over two or more of the following resins: cation exchange resin SP-Sepharose,

reactive green 19 resin, reactive blue 72 resin, reactive yellow 3 resin, and UDP-agarose. Proteins obtained from selected fractions from these columns were treated with trypsin to generate peptides, and the amino acid sequence of the peptides identified by liquid chromatography-tandem mass spectrometry. The amino sequence thus generated was used to screen the *Arabidopsis* gene/protein database. Thirty unique proteins were solely identified in the GalAT-containing fractions (i.e. not present in fractions not containing GalAT activity). Among the 30 unique proteins that co-purified with GalAT activity, two proteins (designated JS33 and JS36) were initially identified as *Arabidopsis* putative GALAT proteins/genes based on their having at least one predicted transmembrane domain and since they contained a predicted glycosyltransferase domain (see CAZy database; http://afmb.cnrs-mrs.fr/CAZY/index.html).

These two genes, along with another *Arabidopsis* gene with high sequence similarity to JS36 (designated JS36L for JS36-like) (see below) were either cloned by RT-PCR (JS36) using mRNA from *Arabidopsis* flower and stem tissue, or a cDNA clone was obtained from the Arabidopsis Biological Resource Center (JS33 and JS36L). The proteins encoded by these genes each have a predicted single transmembrane domain (Table III). The genes were truncated to remove their N-terminal region including all or most of the predicted transmembrane domain (see Table III), and the truncated genes were inserted into a mammalian expression vector pEAK10 (Edge BioSystems as modified by Kelley Moremen lab, CCRC) containing an N-terminal heterologous signal sequence (targeting the protein for secretion into the medium), a polyhistidine (HIS) tag, and two influenza hemagglutenin (HA) epitopes (useful for immunoabsorption).

Table III. *Predicted characteristics of JS36, JS33 and JS36L proteins.* Predictions were made using information from the NCBI database and the SOSUI (Classic & Membrane Prediction program) at BCM Search Launcher site (http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html).

Gene	NCBI protein ID	# amino acids	MW (kd)	pI	Predicted transmembrane domain	Truncated protein
At3g61130 (JS36)	NP_191672	673	77.4	9.95	$^{N}22\text{-}44^{C}$	$^{N}42\text{-}673^{C}$
At2g38650 (JS33)	NP_565893	619	69.7	8.63	$^{N}23\text{-}45^{C}$	$^{N}44\text{-}619^{C}$
At5g47780 (JS36-like)	*NP_568688*	616	71.1	9.26	$^{N}6\text{-}22^{C}$	$^{N}26\text{-}616^{C}$

The truncated forms of JS33, JS36 and JS36L, and the vector alone, were transiently expressed in human embryonic kidney cells (HEK293 cells) for 46 hours. Since the translational fusion proteins constructed contained two copies of the HA epitope, the culture medium was collected and a portion was treated with a mouse anti-HA IgG1 bound either to Protein A Sepharose or Protein G Sepharose. The immunoadsorbed protein was assayed for GalAT activity using UDP-[^{14}C]GalA and a mixture of OGA acceptors. Figure 8 shows that the JS36 construct expressed a protein exhibiting GalAT activity. These studies establish that JS36 is a GalAT and thus we designated the gene *GALAT1.*

As mentioned above, analysis of the amino acid sequence of GALAT1 shows that the expressed protein contains one transmembrane domain. This is in agreement with the GalAT activity being membrane bound in all species tested (see Mohnen *et al.* (2002)[9]. Furthermore, the predicted topology of GALAT1 is that of a type-II membrane protein, in agreement with our previous determination that the catalytic site of pea GalAT lies in the lumen of the Golgi. Type-II membrane proteins have a short N-terminal cytosolic tail, a transmembrane region, a stem region, and a C-terminal catalytic domain[16].

GALAT1 is a member of the Glycosyltransferase Family 8 in the CAZy database [database of putative and proven carbohydrate modifying enzymes that currently contains 61 different proposed glycosyltransferase families (http://afmb.cnrs-mrs.fr/CAZY/index.html)[66,67]]. The presence of GALAT1 in Family 8 is in agreement with our demonstrated activity of GALAT1 as an α1,4-galacturonosyltransferase, since Family 8 is a family of proposed retaining glycosyltransferases and GALAT1 is a retaining enzyme, i.e., the α-configuration in the substrate UDP-α-GalA is retained in the product α1,4-linked-galacturononan (HGA).

GALAT is expressed in multiple *Arabidopsis* tissues at multiple times during development. We base this on our RT-PCR analysis of RNA from *Arabidopsis* flower, root, stem and leaf tissue (Figs. 6A and 6B) showing that GALAT1 is expressed in all these tissues, and based on the 18 EST entries for this gene in the TAIR database (http://www.arabidopsis.org/) indicating that GALAT1 is expressed in developing seed, green siliques, roots and above ground organs.

Identification of the *GALAT1* Gene Family

A standard protein blast and a PSI Blast of the NCBI protein database using the GALAT1 (JS36) amino acid sequence reveal that *GALAT1* is a member of a at least 15 member *GALAT* gene family in *Arabidopsis* (*see* Table IV). The genes selected for this family have at least 30% amino acid identity and at least 50% amino acid similarity based on the PSI Blast. We further compared these genes along their entire coding sequences with JS36 using a Pairwise BLAST (Table IV) and show that this family of genes has at least 34% identity and at least 52% similarity to JS36 in the portion of the genes C-terminal to the membrane spanning domain. This identity is comparable to the 37-54% identity shared among the proposed ten member *Arabidopsis* fucosyltransferase gene family (AtFU1-10)[71].

Mutant studies provide further evidence that the GalAT family encodes GalATs involved in pectin synthesis. We recently used seed received from *Arabidopsis* T-DNA mutant collection (SIGnAL; http://signal.salk.edu/cgi-bin/tdnaexpress) to identify and generate six homozygous *Arabidopsis* GalAT family T-DNA insert mutant lines of several members of the GalAT family. We found that one GalAT family gene At1g06780, when mutated, produces leaves with cell walls that contain reduced amounts of galacturonic acid. Specifically, analysis of walls from homozygous mutant line 073484 revealed that the walls had an 18% reduction in GalA and a concomitant increase in glucose. None of the other sugars changed. Of the three available At1g06780 T-DNA insert lines, no homozygous seed was recovered from mutants where the T-DNA was inserted into an exon. Rather, seed recovered from such lines had a reduced germination rate. In line 073484, however, the T-DNA is inserted in the 5'-UTR, suggesting that it may have a leaky phenotype. The results are consistent with gene At1g06780 encoding a GalAT and with the identification of the gene family as a GalAT gene family. The GalA content of the walls of another *Arabidopsis* mutant (*Quasimodo*) is reduced by 25% and these plants exhibit decreased cell adhesion[55], characteristics consistent with the *Quasimodo* gene encoding a GalAT. *Quasimodo* has 53% amino acid identity and 72% similarity to GALAT1 and the gene affected in Quasimodo (At3g25140) is a member of our proposed GalAT family. There is, however, at present no direct enzymatic evidence that the protein encoded by *Quasimodo* is a functional GalAT.

The conserved amino acids in the *GALAT* gene family are shown in Fig. 7. Glycosyltransferases are expected to contain one or more carboxylates at the catalytic site. At least one of the carboxylates is expected to coordinate a divalent cation associated with the nucleotide-sugar. In many glycosyltransferases the metal coordination involves two carboxylates that are often present as DDx, xDD, or DDD (the so-called "D(x) D" motif)[72].

A PSI Blast against *GALAT1* gene (JS36) further identified10 genes that have high sequence identity (23-29%) and similarity (41-51%) to GALAT1 and form a tight cluster of highly similar genes (55-66% identity/67-77% similarity). A Neighbor Joining Tree of our proposed *Arabidopsis* GalAT Superfamily (i.e. the proposed *GALAT* family and the *GALAT*-Like family), based on a sequence alignment generated by ClustalX[128], is shown in Fig. 9. The 10 *GALAT*-like genes are all significantly smaller, lacking ~200 amino acids in comparison with the *GALAT* family. Nonetheless, they appear to be targeted to the secretory pathway based on annotation of the genes at the *Arabidopsis* Information Resources. All 10 genes appear to be expressed in *Arabidopsis*, since they are represented by one or more ESTs in the *Arabidopsis* EST collection. The *GALAT*-like genes also contain some of the same conserved residues as the GalAT family, namely D-D-----D---L (the predicted "D(x) D" motif) and L------F----------W---GLG----------H—G—KPW. We group the 10 *GALAT*-like genes into a family that encode GalATs directly involved in pectin synthesis or GalATs with, as yet, unidentified glycosylating function.

Table IV. Pairwise sequence alignment between JS36 and the other members of proposed GALAT gene family. The alignment was done using the NCBI Pairwise BLAST and Matrix Blosum62. The % amino acid identity and similarity are shown. In all cases the alignment compares the bulk of the C-terminal portion of the proteins on the carboxy-terminal side of the transmembrane region.

Gene	NCBI protein ID	EMBL protein #	% Identity (#aa identical/#aa)	% Similar amino acids (aa/aa)
GalAT-Family				
***At3g61130 (GALAT1; JS36)	NP_191672	Q9LE59	**100%** (673/673)	**100%** (673/673)
At5g47780 (JS36-like)	NP_568688	Q93ZX7	**63%** (290/458)	**81%** (374/458)
At2g46480	**NP_182171**		**61%** (297/485)	**75%** (365/485)
At4g38270	NP_195540		**55%** (344/620)	**73%** (459/620)
At3g25140 (Quasimodo)	**NP_189150**	Q9LSG3	**53%** (241/450)	**72%** (330/450)
At1g18580	**AAK93644**		**48%** (226/469)	**67%** (317/469)
At3g02350	**NP_566170**	Q9FWA4	**47%** (247/521)	**66%** (350/521)
At2g20810	**NP_565485**	Q93VL7	**46%** (215/462)	**68%** (320/462)
At1g06780	NP_563771	Q9M9Y5	**44%** (204/461)	**63%** (296/461)
At2g30575	NP_850150		**43%** (203/463)	**65%** (309/463)
At3g01040	**NP_186753**	Q9MAB8	**42%** (189/447)	**61%** (227/447)
At5g15470	**NP_197051**	Q9LF35	**42%** (189/443)	**61%** (274/443)
At5g54690	**NP_200280**	Q9FH36	**38%** (169/436)	**60%** (265/436)
At2g38650 (JS33)	NP_565893	Q949N9	**36%** (171/475)	**60%** (286/475)
At3g58790	**NP_191438**	Q9LXS3	**34%** (160/458)	**52%** (247/458)
GalAT-Like Family				
At1g02720	NP_171772		26 (85/316)	44 (143/316)
At1g13250	NP_563925	Q9FX71	23 (86/359)	41 (154/359)
At1g19300	NP_564077	Q9LN68	29 (58/198)	49 (98/198)
At1g24170	NP_173827	O48684	23 (75/322)	41 (136/322)
At1g70090	NP_564983	O04536	27 (64/233)	48 (115/233)
At3g06260	NP_187277	Q9M8J2	29 (52/179)	51 (92/179)
At3g28340	NP_189474	Q9LHD2	28 (56/194)	52 (104/194)
At3g50760	NP_190645	Q9S7G2	24 (76/308)	43 (137/308)
At3g62660	NP_191825	Q9LZJ9	29 (56/191)	51 (99/191)
At4g02130	NP_192122		29 (58/197)	51 (103/197)

The expression of the *GALAT1* gene in transiently transfected mammalian cells as demonstrated herein now allows the production of stably transformed cell lines that produce GALAT1 and experiments aimed at characterizing the mechanism of the enzyme and at determining the role of GalAT1 in pectin synthesis. Specifically, the substrate specificity of GalAT1 will indicate whether it catalyzes only HGA synthesis, or also plays a role in RG-I and RG-II synthesis. Characterization of the kinetics of GalAT1 can clarify whether or not UDP-GalA is both a substrate and an allosteric regulator of the enzyme. Characterization of the mutated GalA1 enzyme can provide information regarding amino acids important in catalysis and substrate binding. The subcellular location of GALAT1 will provide the first framework for where, within the Golgi and plant endomembrane system the complex series of pectin biosynthetic reactions occur. The invention can further be used to generate transgenic plants with modified pectin, which can provide information regarding the role of GALAT1 in pectin synthesis, provide novel biosynthesis acceptors, and provide information about the role of pectin in plant growth and development. This biosynthesis framework allows further identification of GALAT1 binding proteins that would be putative pectin biosynthesis complex members. The results of these studies can serve as the foundation for a full *in vitro* reconstitution of functional pectin synthesis complexes.

GALAT1 has high sequence similarity to 14 other *Arabidopsis* proteins as shown in Table IV and to proteins expressed in other plants. Possible *GALAT1* homologs in other plants are a 68 kd protein expressed in *Cicer arietinum* (chickpea) epicotyls (76% amino acid identity; 87% similarity), a hypothetical protein from *Oryza sativa* (japonica) (59% identify; 75% similarity) and a protein from *Populus alba* (49% identity; 72% similarity). Thus, the results from the study of GALAT1 in *Arabidopsis* can be extended to other plants, including those of high agricultural value.

Heterologous expression of GALAT1

As described above, the media from human embryonic kidney (HEK293) cells transiently infected with recombinant expression vector bearing truncated *GALAT1* expressed GALAT1. Whereas transient expression allowed the

expression of sufficient GALAT to measure GalAT activity, additional expression strategies can be readily devised to produce large quantities of GALAT1 required for further characterization of the enzyme and for antibody production. Since the transiently expressed N-terminal epitope-tagged GALAT1 expressed in mammalian cells was active, one strategy is to produce stably transfected clonal HEK293 lines[75] expressing the same protein. The alternative strategy is to express the full length and N-terminal truncated forms of GALAT1 in the fungal expression system *Pichia pastoris*. These systems were chosen since we and others[56-58] have successfully used them to express plant glycosyltransferases.

For expression in *P. pastoris*, cDNA encoding the entire, and the truncated soluble forms of GALAT can be generated by PCR using gene/vector specific primers. The PCR products are then subcloned into appropriate *Pichia* expression vectors (Invitrogen, Carlsbad, CA) in which the cDNA is inserted downstream from an alcohol oxidase (AOX1) promoter. We have made full length coding sequence constructs for expression in the *Pichia* vector pPIC 3.5. This vector does not contain an epitope tag. One can easily make epitope tagged GALAT1 constructs in the *Pichia* vectors pPICz and pPICzα (Invitrogen) and determine whether functional C-terminal epitope-tagged constructs that do not affect GalAT activity can be recovered. Several studies have demonstrated success of the *Pichia* system[76-82]. Once a high-GALAT1-producing line is recovered, production of large amounts of protein can be carried out in fermentors or spinner flasks.

Characterization of Expressed GALAT1

To begin to address how HGA is synthesized, the kinetics, substrate specificity, and structure of the purified recombinantly expressed GALAT1 can be determined and compared to the solubilized membrane-bound *Arabidopsis* GALAT purified by immunoadsorption using the polyclonal-antiGALAT1 (see below). Although the characteristics of GalAT1 are consistent with the enzyme being the/a catalytic subunit of the HGA synthase, GALAT1 could be a GalAT involved in RG-II or RG-I synthesis. For example, GalAT could represent an RG-I:GalAT that initially elongates HGA by a single GalA and then waits for a required NDP-Rha to start RG-I backbone synthesis. The kinetics of purified and recombinantly expressed GALAT1 for UDP-GalA and a size range of homogalacturonan and pectin

acceptors can be determined. The effect of other nucleotide-sugars and oligosaccharide substrates on GalAT can also be tested to identify activators and inhibitors.

The expressed full length and truncated enzymes can be assayed in a reaction buffer in the presence, and absence, respectively, of Triton X-100. The kinetics of the enzyme for UDP-GalA can be carried out in a total of 1 μM to 80 mM UDP-GalA + UDP-[^{14}C]GalA. We routinely synthesize UDP-[^{14}C]GalA either by the 4-epimerization of UDP-[^{14}C]GlcA[1] or oxidation of UDP-[^{14}C]Gal[84] since UDP-[^{14}C]GalA is not commercially available. The effect of different acceptors on GALAT1 activity can be conducted using 100 μM UDP-GalA and 0.1-100 μg acceptor/ 30 μl reaction. The acceptors to be tested include HGA oligosaccharides (oligogalacturonides) of degrees of polymerization ranging from 2-16, polygalacturonic acid, commercially available citrus pectin of ~30, 60 and 90% esterification, RG-I and RG-II. The products made using the different acceptors can be characterized[2,3]. If RG-I is shown to serve as an acceptor, RG-I backbone fragments that have a GalA or a Rha at the non-reducing end can be used to determine acceptor specificity. The acceptors can be tested using multiple assays including the precipitation assay[2] and a filter assay[63]. The enzymes can also be tested for the effect of pH, temperature, reducing agents, divalent cations and salts on enzyme activity and product structure.

Characteristics of the recombinant truncated GALAT1 can be compared to the GALAT1 solubilized from *Arabidopsis* membranes by immunoadsorption of the solubilized GALAT1 using anti-GALAT1 antibody (see section below) bound to Protein A or G Sepharose, or by coupling the anti-GALAT1 antibodies to 3M-Emphaze resin[86] and using the resin used to purify GALAT1 from solubilized *Arabidopsis* enzyme. If the characteristics of the immunoadsorbed *Arabidopsis* GALAT1 are different from those of the recombinant truncated GALAT1, the immunoadsorbed GALAT1 can be analyzed by LC tandem mass spectrometry to determine if additional proteins are immunoadsorbed with the *Arabidopsis* solubilized GALAT1 that may have modified the activity (e.g. a heteromeric complex).

The recombinant GALAT1 and the GALAT1 immunoadsorbed-from *Arabidopsis* solubilized membranes can also be treated with *N*-glycanase to determine if they are *N*-glycosylated. To determine if they are O-glycosylated, the proteins can be exhaustively treated with *N*-glycanase, the released oligosaccharides removed, and the resulting protein analyzed by TMS methylation analysis to determine the glycosyl residue composition of any carbohydrates still attached to the protein. Any oligosaccharide released by the N-glycanase treatment can also be analyzed by TMS methylation. The results of these experiments would indicate whether the native *Arabidopsis* GalAT is glycosylated and whether the recombinant forms have the same or different glycosylation pattern. Changes in glycosylation could affect GalAT1 enzyme activity and/or substrate binding. GALAT1 is predicted to have 5 or 6 *N*-glycosylation sites (NetNGlyc 1.0 Prediction; http://www.expasy.org/sitemap.html).

As mentioned above, we have found that membrane-bound and solubilized GalAT activity in tobacco and radish has unusual apparent biphasic kinetics. Thus, we are particularly interested in determining if the expressed GALAT1 shows the same kinetics, including possible allosteric regulation by UDP-GalA. One can test for possible multimeric structure by determining the mass of the enzyme by size exclusion chromatography and comparing these with the mass obtained by SDS-PAGE. The possibility that GALAT1 exists as a heteromultimer can be tested by mixing expressed recombinant GALAT1 with solubilized *Arabidopsis* enzymes and immunoadsorbing GALAT1 and proteins bound to it using either an anti-GALAT1 antibody or an anti-HA epitope antibody (see previous section).

Production of a series of mutated GALAT1 proteins by site-directed mutagenesis

As discussed above, there are 45 conserved amino acids in GALAT1 among the 15 members of the *GALAT* family. To determine the role of these residues in substrate/acceptor binding and/or catalysis, each amino acid is systematically mutated using site-directed mutagenesis. The effect of these mutations on GALAT1 specific activity, and where warranted, on Km, Vmax, and acceptor specificity (i.e. OGA, RG-I and RG-II) and product size (i.e. enzyme processivity) is determined.

Production and use of antibodies

Anti-GalAT antibodies are necessary for the immunocytochemistry experiments, to immunopurify solubilized GALAT1 from *Arabidopsis*, and to select proteins that potentially bind to GALAT1 and may function in pectin biosynthetic enzyme complexes. A skilled artisan can generate anti-GalAT antibodies using the nucleic acid or amino acid sequences disclosed herein. This can be accomplished by employing the heterologously expressed truncated or full-length GALAT1. Alternatively, a small peptide derived from the GALAT1 sequence can be synthesized and used to generate anti-GALAT1 antibodies. One can generate either polyclonal or monoclonal antibodies. Such antibodies are useful for a range of types of experiments, including subcellular immunocytochemistry, immunoprecipitation/adsorption, and enzyme activity inhibition studies. Monoclonal or polyclonal antibodies, specifically reacting with a protein of interest can be made by methods well known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1996) *Monoclonal Antibodies: Principles and Practice*, 3rd ed., Academic Press, San Diego, CA, and Ausubel *et al.* (1993) *Current Protocols in Molecular Biology*, Wiley Interscience/Greene Publishing, New York, NY.

Subcellular localization of GALAT1

All available data, including the localization of the catalytic domain of GalAT in the Golgi lumen[7], suggest that pectin is synthesized in the Golgi and transferred via vesicles to the wall. However, it is not known how the different glycosyltransferases function to make specific pectin structures. We predict that different glycosyltransferases are localized in a sequential manner to different cisternae of the Golgi[22,91] in an order indicative of the order in which pectin is synthesized as it moves from the cis, through the medial and to the trans Golgi. Evidence from both animal[92,93] and plants[94] suggests that, either individually or in combination, the transmembrane domain (i.e. the bilayer thickness model[95]), the N- or C-terminal sequences flanking the transmembrane domain, and/or the lumenal domain (i.e. the 'kin recognition model'[96]) contribute to localization of proteins within the Golgi system. The anti-GalAT antibodies generated as described above can be used to determine the subcellular localization of GALAT1 within the Golgi in order

to provide additional information on the role of GalAT1 in pectin synthesis. For example, a location of GALAT1 in the cis and medial Golgi cisternae would be consistent with a function of GALAT1 in HGA synthesis, while a localization primarily in the late medial or trans Golgi would be more suggestive of a role in RG-I or possible RG-II synthesis. It should be noted that such subcompartment localization studies, while important and novel for the pectin biosynthetic enzymes, are also novel in any species since the "precise location of only a small number of the glycosyltransferase proteins within the Golgi apparatus have been determined"[93]. Anti-GALAT1 antibodies can be used to identify where in the Golgi GALAT1 is localized by, for example, immunogold label of thin sections from Arabidopsis[97, 91,98, 99] including both developing *Arabidopsis* seedlings and growing suspension cultures which have cells actively making wall.

Use of mutants and RNAi to generate and characterize *GALAT1* and GalAT gene Superfamily knockouts.

Double-stranded RNA-mediated interference (RNAi) is a method to study the function of genes in plants[100]. Transgenic plants harboring an RNAi construct often have reduced expression of the gene-specific mRNA. The resulting plants may display either complete gene silencing, thus having a knockout phenotype, or a partial "knockout" phenotype due to 'leaky' expression. The RNAi approach should allow the suppression of *GALAT1* expression and a reduction or loss of GALAT1. This enables one to elucidate the function of GALAT in pectin synthesis and in the plant. Simultaneously, the sequence-indexed T-DNA insertion mutants listed in the Salk Institute Genomic Analysis Laboratory (SIGnAL) *Arabidopsis* T-DNA mutant collection (http://signal.salk.edu/cgi-bin/tdnaexpress) can be monitored to determine if any T-DNA insert lines for GALAT become available. If so, the seed can be obtained and the mutants generated therefrom can be characterized (as described above).

The putative pectin biosynthesis mutants can aid in the identification of gene function in two ways. The visible phenotypes of the mutants can provide information on the biological function of the gene (if there is no redundancy in gene function) by demonstrating when during growth and development the particular

gene product is needed (as shown above). Structural analysis of the pectin in the mutant walls can provide information about the specific enzyme activity of the gene in pectin synthesis (as shown above).

Of particular importance regarding pectin synthesis, the cell walls are isolated and analyzed for glycosyl residue composition (see above) and linkage to provide information about the possible role of GALAT1 in pectin synthesis.

Identification of the members of HGA biosynthetic complexes.

There is growing evidence that glycoconjugates are synthesized by complexes of glycosyltransferases and other types of proteins[102]. For example, ganglioside synthesis occurs via a tightly regulated formation of multiple glycosyltransferase complexes[102]. Thus, any protein members of HGA biosynthetic complexes can be isolated by immunoadsorbing such proteins bound to GALAT1 using anti-GALAT1 antibodies or anti-HA epitope antibodies. The immunoadsorbed proteins can be identified by SDS-PAGE, removed from the gel, and their amino acid sequence determined by LC-tandem mass spectrometry. The amino acid sequences thus obtained can then be used to search the available protein databases for their identities.

Characterization of mutant phenotypes and bulking up of seed.

A person of ordinary skill in the art can use mutant seeds to probe gene function. For example, the initial mutant seed (often a segregating T3 line, see http://signal.salk.edu/tdna_FAQs.html) can be grown and selfed to increase the seed stock (T4). Multiple plants from T4 seed can be grown and the presence of, for example) the T-DNA insert determined by PCR of plant genomic DNA using a T-DNA primer and a gene specific primer. The same DNA can be analyzed with gene specific primers that should span the T-DNA insertion site. These analyses should indicate whether the given plant contains a T-DNA insert and if so, whether it is homozygous or heterozygous for the mutation. If necessary, Southern blotting and hybridization with the specific genes can be used to determine if the gene contains the expected T-DNA insert. Seed homozygous for the T-DNA insertion (when not lethal) or heterozygous (when no viable TDNA homozygous plants are obtained) can be selfed to amplify the seed and, for heterozygous plants, to test for

segregation of any phenotype or T-DNA insert. Plants can be scored as heterozygous or homozygous by PCR analysis of the T-DNA insert and by any visible phenotype. Homozygous or heterozygous plants can be used for growth phenotype and cell wall analysis. The seed can also be crossed with wild type Columbia and then selfed to eliminate the possibility that the lines contain an unexpected mutation or additional T-DNA insert(s).

Growth Phenotype analysis

Several growth parameters of the mutant and wild type plants are recorded to yield a general phenotypic characterization of the mutant plants.[134]

Analysis of Cell Walls

Homozygous or heterozygous plants are grown and analyzed for wall composition and linkage. Cell walls can, for example. be prepared as alcohol insoluble residues (AIRs) from WT and (homozygous) mutant *Arabidopsis* plant tissues[135]. AIRs are prepared by homogenizing leaves and stems (from soil-grown plants) and roots (from liquid-cultured plants) in aquous 80% EtOH followed by washes with absolute EtOH, chloroform-methanol, and acetone. Separate fractions containing RG-I, RG-II and oligogalacturonides can be obtained by size-exclusion chromatography (SEC) and ion exchange chromatography of the material solubilized from the cell walls by treatment with pectin methyl esterase (PME) and *endo*-polygalacturonase (EPG). The yields, glycosyl residue compositions, and glycosyl linkage compositions of each fraction can be determined[27].

The nucleotide and amino acid sequences of the fifteen *GALAT* gene family members are shown as follows.

Sequence #1 (SEQ ID NO:1)

Gene name: At3g61130
GeneBank accession # for reference: NM_115977 GI:18411855
Nucleotide sequence of Sequence #1:
Positions 1-2022 of CDS of NM_115977.

```
   1 atggcgctaa agcgagggct atctggagtt aaccggatta gaggaagtgg tggtggatct
  61 cgatctgtgc ttgtgcttct catatttttc tgtgtttttg cacctctttg cttctttgtt
 121 ggccgaggag tgtatatcga ttcctcaaat gattattcaa ttgtttctgt gaagcagaat
 181 cttgactgga gagaacgttt agcaatgcaa tctgttagat ctcttttctc gaaagagata
 241 ctagatgtta tagcaaccag cacagctgat ttgggtcctc ttagccttga ttcttttaag
 301 aaaaacaatt tgtctgcatc atggcgggga accggagtag acccctcctt tagacattct
 361 gagaatccag caactcctga tgtcaaatct aataacctga atgaaaaacg tgacagcatt
 421 tcaaaagata gtatccatca gaaagttgag acacctacaa agattcacag aaggcaacta
 481 agagagaaaa ggcgtgagat gcgggcaaat gagttagttc agcacaatga tgacacgatt
 541 ttgaaactcg aaaatgctgc cattgaacgc tctaagtctg ttgattctgc agtccttggt
 601 aaatacagta tttggagaag agaaaatgag aatgacaact ctgattcaaa tatacgcttg
 661 atgcgggatc aagtaataat ggctagagtc tatagtggga ttgcaaaatt gaaaaacaag
 721 aacgatttgt tacaagaact ccaggcccga cttaaggaca gccaacgggt tttgggggaa
 781 gcaacatctg atgctgatct tcctcggagt gcgcatgaga aactcagagc catgggtcaa
 841 gtcttggcta aagctaagat gcagttatat gactgcaagc tggttactgg aaagctgaga
 901 gcaatgcttc agactgccga cgaacaagtg aggagcttaa agaagcagag tacttttctg
 961 gctcagttag cagcaaaaac cattccaaat cctatccatt gcctatcaat gcgcttgact
1021 atcgattact atcttctgtc tccggagaaa agaaaattcc ctcggagtga aaacctagaa
1081 aaccctaatc tttatcatta tgccctcttt tccgacaatg tattagctgc atcagtagtt
1141 gttaactcaa ccatcatgaa tgccaaggat ccttctaagc atgtttttca ccttgtcacg
1201 gataaactca atttcggagc aatgaacatg tggttcctcc taaacccacc cggaaaggca
1261 accatacatg tggaaaacgt cgatgagttt aagtggctca attcatctta ctgtcctgtc
1321 cttcgtcagc ttgaatctgc agcaatgaga gagtactatt ttaaagcaga ccatccaact
1381 tcaggctctt cgaatctaaa atacagaaac ccaaagtatc tatccatgtt gaatcacttg
1441 agattctacc tccctgaggt ttatcccaag ctgaacaaaa tcctcttcct ggacgatgac
1501 atcattgttc agaaagactt gactccactc tgggaagtta acctgaacgg caaagtcaac
1561 ggtgcagtcg aaacctgtgg ggaaagtttc cacagattcg acaagtatct caacttttcg
1621 aatcctcaca ttgcgaggaa cttcaatcca aatgcttgtg gatgggctta tggaatgaac
1681 atgttcgacc taaaggaatg gaagaagaga gacatcactg gtatatacca caagtggcaa
1741 aacatgaatg agaacaggac actatggaag ctagggacat tgccaccagg attaataaca
1801 ttctacggat taacacatcc cttaaacaag gcgtggcatg tgctgggact tggatataac
1861 ccgagtatcg acaagaagga cattgagaat gcagcagtgg ttcactataa cgggaacatg
1921 aaaccatggt tggagttggc aatgtccaaa tatcggccgt attggaccaa gtacatcaag
1981 tttgatcacc catatcttcg tcgttgcaac cttcatgaat aa
```

Amino Acid Sequence of Sequence #1: (SEQ ID NO:2)
GeneBank ID# NP_191672
Positions 1-673 of NP_191672.

1 malkrglsgv nrirgsgggs rsvlvlliff cvfaplcffv grgvyidssn dysivsvkqn
61 ldwrerlamq svrslfskei ldviatstad lgplsldsfk knnlsaswrg tgvdpsfrhs
121 enpatpdvks nnlnekrdsi skdsihqkve tptkihrrql rekrremran elvqhnddti
181 lklenaaier sksvdsavlg kysiwrrene ndnsdsnirl mrdqvimarv ysgiaklknk
241 ndllqelqar lkdsqrvlge atsdadlprs aheklramgq vlakakmqly dcklvtgklr
301 amlqtadeqv rslkkqstfl aqlaaktipn pihclsmrlt idyyllspek rkfprsenle
361 npnlyhyalf sdnvlaasvv vnstimnakd pskhvfhlvt dklnfgamnm wfllnppgka
421 tihvenvdef kwlnssycpv lrqlesaamr eyyfkadhpt sgssnlkyrn pkylsmlnhl
481 rfylpevypk lnkilflddd iivqkdltpl wevnlngkvn gavetcgesf hrfdkylnfs
541 nphiarnfnp nacgwaygmn mfdlkewkkr ditgiyhkwq nmnenrtlwk lgtlppglit
601 fyglthplnk awhvlglgyn psidkkdien aavvhyngnm kpwlelamsk yrpywtkyik
661 fdhpylrrcn lhe

Sequence #2 (SEQ ID NO:3)

Gene name: At2g38650
GeneBank accession # for reference: NM_129422 GI:30687590
Nucleotide sequence of Sequence #2:
Positions 1-1860 of CDS of NM_129422

1 atgaaaggcg gaggcggtgg tggaggaggt ggtggcggag gaaaacgccg gtggaaagtt
61 ctggtgattg gagttttggt tcttgttatt ctttctatgc ttgttcctct tgctttctta
121 ctcggtcttc acaatggctt tcactctcct ggatttgtca ctgttcaacc ggcttcttca
181 tttgagagct ttaccagaat caatgctact aagcatacac agagagatgt atccgaacgg
241 gtcgatgagg ttcttcaaaa aatcaatcca gttcttccca agaaaagcga cataaacgtg
301 ggttccagag atgtgaatgc aacaagcggc actgattcta aaaaaagagg attaccagtg
361 tccccaactg ttgttgccaa tccaagccct gcaaataaaa caaaatcgga agcctcatat
421 acaggtgttc agaggaaaat agtaagtggt gatgaaactt ggagaacttg tgaagtgaaa
481 tatgggagct actgcctctg gagggaggaa aataaggaac caatgaaaga tgccaaggtg
541 aagcaaatga aggaccagct gtttgtggct agagcatact atcccagtat tgctaaaatg
601 ccttctcaaa gcaagttgac tcgggatatg aaacagaata tccaagagtt tgagcgtatt
661 cttagtgaaa gttctcaaga tgctgacctt ccaccacagg ttgataaaaa gttgcagaag
721 atggaagctg taattgcaaa ggcaaagtct tttccagtcg actgtaacaa tgttgacaag
781 aaattgagac agatccttga tttgactgag gatgaagcta gtttccacat gaaacagagt
841 gtgttcctct accagcttgc agtacagaca atgcctaaga gtcttcattg cttgtcaatg
901 cgactaactg tggaacattt caagtcagat tcacttgagg atcccattag tgagaaattt
961 tcagatccct cattacttca ctttgttatc atctccgata atatactagc atcgtccgtt
1021 gtgatcaact caacggttgt acatgcaagg gacagtaaaa actttgtttt ccatgtactg
1081 acagacgagc agaattactt tgcaatgaaa caatggttta ttaggaatcc ttgcaaacaa
1141 tcaactgttc aagtattgaa cattgaaaaa ctcgagctgg acgattctga tatgaaactg
1201 tctttgtctg cggagttccg tgtttccttc cccagtggtg acctttttggc gtctcaacag
1261 aatagaacac actacttatc ccttttctct caatctcact atcttcttcc caaattattt
1321 gacaaattgg agaaggttgt gattctggat gatgacgttg tagtccagcg agacttatct
1381 ccccttttggg accttgatat ggaagggaaa gtgaatggcg ctgttaagtc gtgcactgtg

1441 agattgggtc agctaaggag tctcaagaga ggaaattttg ataccaatgc ttgtctctgg
1501 atgtctggtt tgaatgtcgt tgatcttgct agatggaggg cattgggtgt ttcagaaacc
1561 tatcaaaaat attataaaga gatgagtagt ggagatgagt cgagcgaagc aattgcattg
1621 caggcaagct tgctcacatt tcaagaccaa gtatatgctc ttgacgacaa atgggctcta
1681 tcagggcttg gttatgacta ctacatcaat gcacaagcca taaaaaacgc agccatattg
1741 cactataacg ggaacatgaa gccgtggctt gagctgggaa tcccaaatta caaaaactat
1801 tggagaaggc atctgagtcg ggaagatcgg ttcttgagtg actgtaacgt gaatccttga

Amino Acid Sequence of Sequence #2: (SEQ ID NO:4)
GeneBank ID# NP_565893
Positions 1-619 of NP_565893.

1 mkgggggggg ggggkrrwkv lvigvlvlvi lsmlvplafl lglhngfhsp gfvtvqpass
61 fesftrinat khtqrdvser vdevlqkinp vlpkksdinv gsrdvnatsg tdskkrglpv
121 sptvvanpsp anktkseasy tgvqrkivsg detwrtcevk ygsyclwree nkepmkdakv
181 kqmkdqlfva rayypsiakm psqskltrdm kqniqeferi lsessqdadl ppqvdkklqk
241 meaviakaks fpvdcnnvdk klrqildlte deasfhmkqs vflyqlavqt mpkslhclsm
301 rltvehfksd sledpisekf sdpsllhfvi isdnilassv vinstvvhar dsknfvfhvl
361 tdeqnyfamk qwfirnpckq stvqvlniek lelddsdmkl slsaefrvsf psgdllasqq
421 nrthylslfs qshyllpklf dklekvvild ddvvvqrdls plwdldmegk vngavksctv
481 rlgqlrslkr gnfdtnaclw msglnvvdla rwralgvset yqkyykemss gdesseaial
541 qaslltfqdq vyalddkwal sglgydyyin aqaiknaail hyngnmkpwl elgipnykny
601 wrrhlsredr flsdcnvnp

Sequence #3 (SEQ ID NO:5)

Gene name: At5g47780
GeneBank accession # for reference: NM_124152 GI:30695292
Nucleotide sequence of Sequence #3:
Positions 1-1851 of CDS of NM_124152.

1 atgatggtga agcttcgcaa tcttgttctt ttcttcatgc tcctcaccgt cgttgctcat
61 atccttctct acaccgatcc cgctgcctcc ttcaagaccc ccttttctaa acgcgatttc
121 ctcgaggacg taaccgcctt gactttcaat tccgatgaga atcgtttgaa tcttcttcct
181 cgggaatctc ccgctgtgct cagaggagga ctcgtcggtg ctgtctattc cgataagaat
241 tcacggcggc tagaccaatt gtctgctcga gttctttccg ccaccgacga tgatactcac
301 tcacatactg acatttccat caaacaagtc actcatgatg cagcctcaga ctcgcatatt
361 aatagggaaa atatgcatgt tcaattgacc caacaaacct ctgaaaaagt tgatgagcaa
421 ccagagccta atgcttttgg agctaagaaa gatactggaa acgtgttgat gcctgatgct
481 caagtgaggc atcttaaaga tcagcttatt agggcaaagg tttatctttc ccttccatct
541 gcaaaggcca atgctcattt tgtgagagag cttcgactcc gtattaaaga agttcaacgg
601 gcacttgcag atgcctccaa ggattcggat ctgccaaaga ctgctataga aaagctaaaa
661 gcaatggagc aaacactggc caaaggcaag cagatccaag atgactgttc tacagtggtc
721 aagaagctac gtgctatgct ccactccgca gatgagcagc tacgggtcca taagaagcaa
781 accatgtttt tgactcaatt gactgctaag accattccta aaggacttca ctgccttcct
841 ctgcgcctca ctacagacta ttatgcttta aattcatctg aacaacaatt tccaaatcag
901 gagaaactag aagatactca gctgtatcac tatgcccttt tctctgataa tgttttggct
961 acgtcagttg ttgttaactc taccataacc aatgcaaagc atcccttaaa gcatgtcttc
1021 cacatcgtca cagacagact caattatgcg gcaatgagga tgtggttcct ggacaatcca

1081 cctggcaaag ccaccatcca ggttcagaat gttgaagaat ttacatggct gaattcaagc
1141 tacagtcccg ttctcaaaca gcttagttct agatcgatga tagattatta cttcagagcc
1201 caccatacaa attcagacac caacttgaag ttccggaatc caaaatactt atcgatcctt
1261 aatcatcttc gtttttactt gcctgagatc tttcccaagc tcagcaaagt gctcttcttg
1321 gatgatgata tagttgtgca gaaggacctt tctggtcttt ggtcagttga tctgaaaggt
1381 aatgttaacg gtgctgtaga gacgtgtggg gaaagctttc atcgctttga ccgttatctg
1441 aacttctcaa atccactcat ttccaagaac tttgaccctc gagcttgtgg ttgggcgtat
1501 ggtatgaatg tctttgatct ggatgaatgg aagaggcaaa acatcacaga agtttatcat
1561 cgatggcagg atctgaatca agaccgagaa ttgtggaagc tagggacgtt gccgcctggt
1621 ctaatcacat tttggagacg aacatatccg ctagaccgga aatggcacat actagggctt
1681 ggatacaacc cgagtgtgaa ccaaagggat attgagaggg cagccgtgat acactataat
1741 ggcaacctca aaccatggct agagattggg attccaagat acagaggctt ctggtcaaag
1801 catgtagact atgagcacgt ttatctcaga gaatgcaaca tcaatcctta g

Amino Acid Sequence of Sequence #3: (SEQ ID NO:6)
Genebank ID# NP_568688
Positions 1-616 of NP_568688.

1 mmvklrnlvl ffmlltvvah illytdpaas fktpfskrdf ledvtaltfn sdenrlnllp
61 respavlrgg lvgavysdkn srrldqlsar vlsatdddth shtdisikqv thdaasdshi
121 nrenmhvqlt qqtsekvdeq pepnafgakk dtgnvlmpda qvrhlkdqli rakvylslps
181 akanahfvre lrlrikevqr aladaskdsd lpktaieklk ameqtlakgk qiqddcstvv
241 kklramlhsa deqlrvhkkq tmfltqltak tipkglhclp lrlttdyyal nsseqqfpnq
301 ekledtqlyh yalfsdnvla tsvvvnstit nakhplkhvf hivtdrlnya amrmwfldnp
361 pgkatiqvqn veeftwlnss yspvlkqlss rsmidyyfra hhtnsdtnlk frnpkylsil
421 nhlrfylpei fpklskvlfl dddivvqkdl sglwsvdlkg nvngavetcg esfhrfdryl
481 nfsnpliskn fdpracgway gmnvfdldew krqnitevyh rwqdlnqdre lwklgtlppg
541 litfwrrtyp ldrkwhilgl gynpsvnqrd ieraavihyn gnlkpwleig ipryrgfwsk
601 hvdyehvylr ecninp

Sequence #4 (SEQ ID NO:7)

Gene name: At1g06780
GeneBank accession # for reference: NM_100555 GI:30679825
Nucleotide sequence of Sequence #4:
Positions 1-1770 of CDS of NM_100555.

1 atgaaacaaa ttcgtcgatg gcagaggatt ttgatcctcg ctctgctatc gatatcagta
61 ttcgctccgc ttattttcgt atcgaatcgg cttaagagca tcactcccgt tggtcgtaga
121 gaatttattg aagagttatc caaaattaga ttcacgacaa atgaccttag acttagcgct
181 attgaacatg aggatggaga aggcttgaag gggccaaggc tcattctctt caaggatggg
241 gagtttaatt cgtctgctga aagtgatggt ggtaatactt acaaaaacag ggaagaacaa
301 gtgattgttt cacagaagat gacagttagc tctgatgaaa agggtcaaat tctaccaaca
361 gtcaaccaac ttgctaataa aacggatttc aagccccctt tatctaaggg tgaaaagaac
421 acaagggttc agcccgacag agcaacagat gtgaaaacga aggagatcag agacaaaatt
481 attcaagcta aagcctacct gaatttcgct ccacctggaa gtaactctca agttgtgaag
541 gagttgagag gtcggctgaa agagctggaa cggtctgttg gtgatgcaac aaaggacaag
601 gacttatcaa agggcgctct ccgcagggtg aagcccatgg aaaatgtgtt atataaggct

661 agtcgtgtct ttaacaattg ccctgccatc gctaccaaac tccgtgccat gaattataac
721 acagaagaac aagttcaggc gcagaaaaat caagcagcgt atctaatgca gcttgcagca
781 aggaccaccc caaaagggct tcactgtctc tcaatgcggc tgacatcaga atacttttca
841 ctggatcctg aaaaaaggca gatgcctaac cagcaaaatt attttgacgc taatttcaat
901 cattatgttg tcttctctga caatgttttg gcttcttcag tcgttgttaa ctctacgata
961 tcttcatcaa aggagccaga aagaatagtc ttccatgtcg tgactgattc acttaattac
1021 ccagcaatct caatgtggtt tctgctaaac attcaaagta aagctactat ccaaatccta
1081 aacattgatg atatggatgt cctgcctaga gattatgatc aattactgat gaagcaaaac
1141 tctaatgacc caagattcat ttctacactc aatcacgcac gcttctatct cccggatata
1201 ttcccgggtt tgaacaagat ggtactcttg gaccatgatg tagttgttca aagagattta
1261 agtagactgt ggagcattga tatgaaagga aaggtggttg gagctgtaga gacttgtctt
1321 gaaggtgaat cttcatttcg atcaatgagc acatttatta atttctcaga cacatgggtc
1381 gctgggaaat ttagtcctag agcttgcaca tgggctttcg ggatgaatct aattgatctc
1441 gaagaatgga gaatacggaa gttgacttct acatacataa aatacttcaa cctgggaaca
1501 aagagaccat tgtggaaagc tgggagctta ccaataggtt ggttgacttt ctataggcaa
1561 acattagcat tggacaagag atggcatgtg atggggttag gtcgcgaatc aggagtcaaa
1621 gcggttgaca tcgaacaagc ggcagttata cactacgatg gggtcatgaa gccgtggttg
1681 gacattggaa aagagaatta caaacgttac tggaacatac acgtccctta ccatcacacc
1741 tacttgcaac agtgcaatct tcaagcttga

Amino Acid Sequence of Sequence #4: (SEQ ID NO: 8)
Genebank ID# NP_563771
Positions 1-589.

1 mkqirrwqri lilallsisv faplifvsnr lksitpvgrr efieelskir fttndlrlsa
61 iehedgeglk gprlilfkdg efnssaesdg gntyknreeq vivsqkmtvs sdekgqilpt
121 vnqlanktdf kpplskgekn trvqpdratd vktkeirdki iqakaylnfa ppgsnsqvvk
181 elrgrlkele rsvgdatkdk dlskgalrrv kpmenvlyka srvfnncpai atklramnyn
241 teeqvqaqkn qaaylmqlaa rttpkglhcl smrltseyfs ldpekrqmpn qqnyfdanfn
301 hyvvfsdnvl assvvvnsti ssskeperiv fhvvtdslny paismwflln iqskatiqil
361 niddmdvlpr dydqllmkqn sndprfistl nharfylpdi fpglnkmvll dhdvvvqrdl
421 srlwsidmkg kvvgavetcl egessfrsms tfinfsdtwv agkfspract wafgmnlidl
481 eewrirklts tyikyfnlgt krplwkagsl pigwltfyrq tlaldkrwhv mglgresgvk
541 avdieqaavi hydgvmkpwl digkenykry wnihvpyhht ylqqcnlqa

Sequence #5 (SEQ ID NO:9)

Gene name: At1g18580
GeneBank accession # for reference: AY062444 GI:17064735
Nucleotide sequence of Sequence #5:
Positions 1-1614 of CDS of AY062444.

```
   1 atgaggcggt ggccggtgga tcaccggcgg cgaggtagaa ggagattgtc gagttggata
  61 tggtttctcc ttggttcttt ctctgtcgct ggtttagttc tcttcatcgt tcagcattat
 121 caccatcaac aagatccatc ccagctttta cttgagagag acacgagaac cgaaatggta
 181 tctcctcccc atttaaactt cacggaagag gtcacaagtg cttcctcctt ctctaggcag
 241 ttagcagagc aaatgacact tgccaaagct tatgtgttta tagctaaaga gcataataat
 301 cttcatttag cttgggaatt gagttctaag atcagaagtt gtcagctttt gctttccaaa
 361 gcagctatga gaggacaacc tatttcgttt gatgaggcta aaccgattat tactggtcta
 421 tcagctctta tctacaaggc tcaagatgca cattatgata ttgccaccac tatgatgacc
 481 atgaaatctc acatccaagc acttgaagag cgtgcaaatg cagctactgt tcagaccaca
 541 atatttgggc aattggttgc tgaggcatta ccaaagagcc tccactgttt gacgataaag
 601 ctcacatctg attgggtaac agagccatct cgccatgaac tggcagatga gaacagaaac
 661 tcacctagac ttgtcgacaa caacctctac cacttctgca tcttctcgga caacgtgatt
 721 gccacctcgg ttgttgttaa ttcaactgtc tcgaatgctg atcatccaaa gcagcttgtt
 781 ttccacatag tgacgaatcg agtgagctac aaagctatgc aggcctggtt tctaagtaat
 841 gacttcaagg gctcagcaat agagatcagg agcgtagagg agttttcttg gttgaatgct
 901 tcatattctc ctgttgttaa gcaactgctg gacacagatg caagagctta ctatttcggg
 961 gaacagacaa gtcaagatac gatttccgag ccaaaagtga ggaacccaaa gtacttgtca
1021 ttactgaacc atctcagatt ctacattccg gagatctatc cacagctaga gaagattgtt
1081 ttcctagacg atgatgttgt tgttcagaaa gatttgactc cactcttctc cttggatctg
1141 catggaaacg tcaatggagc tgtggaaaca tgtcttgaag cctttcaccg atattacaag
1201 tatctaaatt tctcgaaccc actcatcagc tcaaagttcg acccacaagc atgtggatgg
1261 gcttttggta tgaacgtttt tgatctgatc gcttggagga atgcaaacgt gactgctcgg
1321 taccattact ggcaagatca gaacagagaa cgaacgcttt ggaaactcgg gacactccct
1381 ccaggtctac tatctttcta tggtctcaca gagccactgg acagaagatg gcatgtcttg
1441 ggtttaggtt acgatgtgaa catcgataac cgtctgatcg aaacagcagc tgtgattcac
1501 tataatggta acatgaagcc ttggctaaag ctggctattg gtaggtataa acctttctgg
1561 ttaaagtttt tgaactcgag ccatccttat ttacaagatt gtgtcacagc ttaa
```

Amino Acid Sequence of Sequence #5: (SEQ ID NO: 10)
Genebank ID# AAK93644 GI:15293067
Positions 1-537 of AAK93644.

```
   1 mrrwpvdhrr rgrrrlsswi wfllgsfsva glvlfivqhy hhqqdpsqll lerdtrtemv
  61 spphlnftee vtsassfsrq laeqmtlaka yvfiakehnn lhlawelssk irscqlllsk
 121 aamrgqpisf deakpiitgl saliykaqda hydiattmmt mkshiqalee ranaatvqtt
 181 ifgqlvaeal pkslhcltik ltsdwvteps rheladenrn sprlvdnnly hfcifsdnvi
 241 atsvvvnstv snadhpkqlv fhivtnrvsy kamqawflsn dfkgsaieir sveefswlna
 301 syspvvkqll dtdarayyfg eqtsqdtise pkvrnpkyls llnhlrfyip eiypqlekiv
 361 fldddvvvqk dltplfsldl hgnvngavet cleafhryyk ylnfsnplis skfdpqacgw
 421 afgmnvfdli awrnanvtar yhywqdqnre rtlwklgtlp pgllsfyglt epldrrwhvl
 481 glgydvnidn rlietaavih yngnmkpwlk laigrykpfw lkflnsshpy lqdcvta
```

Sequence #6 (SEQ ID NO: 11)

Gene name: At2g20810
GeneBank accession # for reference: NM_127647 GI:30681142
Nucleotide sequence of Sequence #6:
Positions 1-1611 of CDS of NM_127647.

```
   1 atgagaagga gaggagggga tagtttccgg agagctggac ggaggaagat ctcgaatgtg
  61 gtatggtggg ttctctctgg tattgccctc ctgctcttct ttctcattct ctccaaagct
 121 ggtcatattg aacctagacc ctctattcct aagcgacgtt accgtaatga caaatttgta
 181 gagggtatga atatgactga ggaaatgttg agtcctactt ccgttgctcg tcaagttaat
 241 gatcagattg ctcttgctaa agcttttgtt gtcattgcta aagaaagtaa gaatcttcag
 301 tttgcttggg acttaagtgc tcagatccgt aactctcagt tgcttttatc gagtgctgct
 361 actaggagaa gtcccttgac tgtcttggaa tctgagtcta ctattcgtga catggctgtt
 421 ttgttatatc aagctcagca gcttcactat gatagtgcta ctatgattat gaggcttaag
 481 gcctcgattc aggctcttga agaacaaatg agttccgtta gcgagaagag ttccaagtat
 541 ggacagattg ctgctgagga agtgcctaag agtctttact gtcttggtgt tcgtctcact
 601 accgaatggt ttcagaattt agacttacag agaactctta aggaaaggag tcgtgttgat
 661 tcgaaactca cggataacag tctctaccat ttctgtgtgt tttccgataa cattattgct
 721 acttctgttg tggttaattc tactgctctc aattccaagg cccctgagaa agttgtgttt
 781 catcttgtga ctaatgagat caactatgct gcaatgaagg cttggttcgc cattaatatg
 841 gacaacctca gaggagtcac tgtggaggtt cagaagttcg aggatttctc atggctgaat
 901 gcttcctatg ttccggtcct caagcagctg caagactctg atacgcaaag ctattatttc
 961 tctggacaca acgatgatgg gcgcactcca atcaaattca ggaaccccaa gtatctttcc
1021 atgctcaacc atcttaggtt ctacatccct gaagtgtttc ctgcgctgaa gaaggtggtc
1081 tttcttgatg atgatgttgt agttcagaag gatctttcat ctctcttttc gatcgattta
1141 aacaaaaatg tgaacggggc tgttgagacc tgcatggaga ccttccaccg ctaccacaag
1201 tacttgaact attctcatcc tctcatacgc tcccactttg atccagatgc gtgtgggtgg
1261 gcgtttggaa tgaacgtctt tgatttagtt gagtggagga agagaaatgt gaccggcata
1321 taccactact ggcaagaaaa aaacgtggac cggaccttat ggaaactggg aacactacct
1381 ccaggacttc tgacatttta cgggttaaca gaggcactag aggcgtcctg gcatatcctg
1441 ggattgggat acacgaatgt ggatgctcgt gtgatagaga aaggagctgt tcttcacttc
1501 aatgggaact taaagccatg gttgaagatc gggatagaga agtacaaacc tttgtgggag
1561 agatacgttg attacacttc tccttttatg caacaatgca attttcattg a
```

Amino Acid Sequence of Sequence #6: (SEQ ID NO: 12)
Genebank ID# NP_565485
Positions 1-536 of NP_565485.

```
   1 mrrrggdsfr ragrrkisnv vwwvlsgial llfflilska ghieprpsip krryrndkfv
  61 egmnmteeml sptsvarqvn dqialakafv viakesknlq fawdlsaqir nsqlllssaa
 121 trrspltvle sestirdmav llyqaqqlhy dsatmimrlk asiqaleeqm ssvsekssky
 181 gqiaaeevpk slyclgvrlt tewfqnldlq rtlkersrvd skltdnslyh fcvfsdniia
 241 tsvvvnstal nskapekvvf hlvtneinya amkawfainm dnlrgvtvev qkfedfswln
 301 asyvpvlkql qdsdtqsyyf sghnddgrtp ikfrnpkyls mlnhlrfyip evfpalkkvv
 361 fldddvvvqk dlsslfsidl nknvngavet cmetfhryhk ylnyshplir shfdpdacgw
 421 afgmnvfdlv ewrkrnvtgi yhywqeknvd rtlwklgtlp pglltfyglt ealeaswhil
 481 glgytnvdar viekgavlhf ngnlkpwlki giekykplwe ryvdytspfm qqcnfh
```

Sequence #7 (SEQ ID NO: 13)

Gene name: At2g30575
GeneBank accession # for reference: NM_179819 GI:30684641
Nucleotide sequence of Sequence #7:
Positions 1-1833 of NM_179819.

```
   1 atgaatcaag ttcgtcgttg gcagaggatt ctgatcctct cgctgctatt gttatctgtt
  61 ttagctccga ttgttttcgt ttcgaatcgg ctcaagagca tcacttccgt cgatagagga
 121 gaattcattg aagaattatc cgacattaca gataagaccg aggatgaact tagacttact
 181 gctattgaac aggacgaaga aggcttgaag gagcctaaac gtattctgca ggatcgagat
 241 tttaattctg tggttttgtc aaattcctct gataaaagta atgatactgt gcagtctaat
 301 gagggagacc aaaaaaactt tctctcagaa gttgataagg gaaataatca caaaccaaag
 361 gaggaacaag cagtttcaca gaaaaccaca gtaagctcga atgcggaggt gaaaatttca
 421 gcaagagata ttcaacttaa tcataaaacg gaattccgac ccccttcaag taagagtgaa
 481 aagaatacaa gggttcaact tgaaagagca acagatgaga gggtaaagga gatcagagac
 541 aaaattatcc aagcgaaagc ctatctgaat ttggccctac ctgggaataa ctcccaaatc
 601 gtaaaggagt tgagagttcg aacgaaagag ctggaacggg ctactggtga tactaccaag
 661 gataaatatt tgccaaagag ctctcctaac agattgaagg ccatggaagt tgcgttatac
 721 aaggtcagcc gtgcctttca caactgccct gccattgcta ccaaactcca agccatgact
 781 tataaaaccg aagaacaagc tcgggcgcag aagaaacaag cagcatattt aatgcagctt
 841 gcagcaagga ctaccccaaa agggcttcat tgtctctcaa tgcggttgac aacagaatat
 901 tttaccctgg atcacgaaaa aaggcagctt ttgcaacaaa gttataatga tcctgatctc
 961 taccattacg tagtcttctc tgacaatgtt ttggcctctt cggttgttgt taactctaca
1021 atctcctcat caaaggaacc ggataaaata gtattccatg tggtgacaga ttcactcaat
1081 tacccagcaa tctcaatgtg gtttttacta aacccaagtg gcagagcttc aatccaaatc
1141 ctaaacattg atgaaatgaa tgtcctgcca ttgtaccatg ctgaattgct gatgaagcaa
1201 aattcaagtg acccaagaat catttcagcg ctcaaccatg cacgcttcta tctcccagat
1261 atcttcccag gtctaaacaa gatcgtactc ttcgatcatg atgtagtagt gcaaagggat
1321 ctaactagac tgtggagcct tgatatgacg gggaaagttg ttggagctgt agagacttgt
1381 cttgaaggtg atccttcata tcgttcgatg gactcattca ttaatttctc agatgcatgg
1441 gtttctcaga aatttgatcc caaggcttgc acttgggcat tcgggatgaa tctatttgat
1501 ctcgaagaat ggagaagaca ggagttgact tctgtatacc tgaaatactt cgacctggga
1561 gtaaaaggac atctgtggaa agcaggggga ttgccagtag gttggttgac ttttttcggg
1621 caaacgtttc cgttggaaaa gagatggaac gtgggtgggt taggtcacga atcaggactc
1681 agggcaagcg acatcgaaca agcagcggtt atacactacg acgggatcat gaaaccatgg
1741 ctggacatcg gtatagacaa gtacaagcgc tactggaaca tacatgtacc ttaccatcac
1801 cctcacttac aacggtgcaa cattcacgat tga
```

Amino Acid Sequence of Sequence #7: (SEQ ID NO: 14)
Genebank ID# NP_850150
Positions 1-610 of NP_850150.

```
   1 mnqvrrwqri lilslllsv lapivfvsnr lksitsvdrg efieelsdit dktedelrlt
  61 aieqdeeglk epkrilqdrd fnsvvlsnss dksndtvqsn egdqknflse vdkgnnhkpk
 121 eeqavsqktt vssnaevkis ardiqlnhkt efrppsskse kntrvqlera tdervkeird
 181 kiiqakayln lalpgnnsqi vkelrvrtke leratgdttk dkylpkspn rlkamevaly
 241 kvsrafhncp aiatklqamt ykteeqaraq kkqaaylmql aarttpkglh clsmrlttey
 301 ftldhekrql lqqsyndpdl yhyvvfsdnv lassvvvnst issskepdki vfhvvtdsln
```

361 ypaismwfll npsgrasiqi lnidemnvlp lyhaellmkq nssdpriisa lnharfylpd
421 ifpglnkivl fdhdvvvqrd ltrlwsldmt gkvvgavetc legdpsyrsm dsfinfsdaw
481 vsqkfdpkac twafgmnlfd leewrrqelt svylkyfdlg vkghlwkagg lpvgwltffg
541 qtfplekrwn vgglghesgl rasdieqaav ihydgimkpw ldigidkykr ywnihvpyhh
601 phlqrcnihd

Sequence #8 (SEQ ID NO: 15)

Gene name: At2g46480
GeneBank accession # for reference: NM_130212 GI:22326493
Nucleotide sequence of Sequence #8:
Positions 1-1587 of NM_130212.

1 atgactgatg cttgttgttt gaagggaaac gaggacaaaa tggttcctcg ttttggtcat
61 ggaacctgga taggaaaagc atttaatgat acaccagaga tgttgcatga aaggagtctg
121 agacaggaaa aaagattgga aagggctaat gagctgatga atgatgatag tctgcaaaag
181 cttgagacgg cagccatggc acgttccaga tctgtcgatt ctgcaccact aggaaactac
241 accatttgga aaaatgaata ccggaggggc aagagttttg aagatatgtt acgtttgatg
301 caagatcaaa tcatcatggc acgagtttac agtggacttg caaagtttac aaacaatctc
361 gccttgcacc aagagataga aacacaacta atgaaactag cttgggagga agaatctact
421 gatattgatc aggagcagag agtacttgac agtataagag acatgggaca aatactggct
481 agagcacacg agcagctata tgaatgcaag ttggtgacaa ataagttgag agcaatgcta
541 caaacagttg aagatgaact cgaaaacgag cagacttata taacgttctt gactcagcta
601 gcttccaagg cactaccaga tgctatccac tgcttgacca tgcgcttgaa tctagagtat
661 catctcctgc ctttaccgat gagaaatttt ccaaggaggg agaatttgga gaatccaaaa
721 ctttaccact acgctctctt ctctgataat gtactggctg catcagttgt tgtcaactcc
781 acagtcatga atgcacagga tccttcaagg catgttttcc accttgtgac tgataagctc
841 aactttggag caatgagtat gtggtttctg ttgaaccctc ctggagaagc gaccatccat
901 gtccaaaggt ttgaagattt tacttggctc aactcatctt actctccagt tttgagtcag
961 ctcgagtcag cagctatgaa gaagttctac ttcaagacag cgaggtctga atcagttgaa
1021 tcaggctcag aaaacctcaa gtaccggtac ccgaaataca tgtcaatgct taaccacctg
1081 aggttctaca tccctaggat cttcccaaag ttggagaaaa tcttgtttgt tgacgatgat
1141 gtggttgttc agaaggattt aactccccta tggtccattg atcttaaagg gaaagtgaat
1201 gaaaactttg atcccaagtt ctgcggatgg gcttatggga tgaacatctt cgacctgaaa
1261 gaatggaaga agaacaacat tacagaaact tatcactttt ggcaaaacct gaacgaaaac
1321 cggactctat ggaaactagg aacattgcca ccagggctca taacgttcta caatctgaca
1381 caaccacttc agagaaaatg gcacttactt ggactgggtt atgataaagg aatcgatgtc
1441 aagaagattg aaagatcagc tgttatacat tacaatggac acatgaaacc atggacagag
1501 atggggataa gcaagtatca gccatattgg acgaagtaca ccaattttga ccatccttac
1561 atctttactt gcaggctgtt tgagtga

Amino Acid Sequence of Sequence #8: (SEQ ID NO: 16)
Genebank ID# NP_182171
Positions 1-528 of NP_182171.

1 mtdacclkgn edkmvprfgh gtwigkafnd tpemlhersl rqekrleran elmnddslqk
61 letaamarsr svdsaplgny tiwkneyrrg ksfedmlrlm qdqiimarvy sglakftnnl
121 alhqeietql mklaweeest didqeqrvld sirdmgqila raheqlyeck lvtnklraml
181 qtvedelene qtyitfltql askalpdaih cltmrlnley hllplpmrnf prrenlenpk

241 lyhyalfsdn vlaasvvvns tvmnaqdpsr hvfhlvtdkl nfgamsmwfl lnppgeatih
301 vqrfedftwl nssyspvlsq lesaamkkfy fktarsesve sgsenlkyry pkymsmlnhl
361 rfyiprifpk lekilfvddd vvvqkdltpl wsidlkgkvn enfdpkfcgw aygmnifdlk
421 ewkknnitet yhfwqnlnen rtlwklgtlp pglitfynlt qplqrkwhll glgydkgidv
481 kkiersavih ynghmkpwte mgiskyqpyw tkytnfdhpy iftcrlfe

Sequence #9 (SEQ ID NO: 17)

Gene name: At3g01040
GeneBank accession # for reference: NM_110969 GI:30678269
Nucleotide sequence of Sequence #9:
Positions 1-1602 of CDS of NM_110969.

1 atgcagcttc acatatcgcc tagcatgaga agcattacga tatcgagcag caatgagttt
61 attgatttga tgaagatcaa agtcgcagct cgtcacatct cttaccgaac tctcttccac
121 actatcttaa tcctcgcttt cttgttacct tttgttttca tcctaaccgc tgttgttacc
181 cttgaaggtg tcaacaagtg ctcctctttt gattgtttcg ggaggcggct aggaccacgt
241 cttcttggta ggatagatga ttcagagcag agactagtta gagattttta caaaattcta
301 aatgaagtaa gcactcaaga aattccagat ggtttaaagc ttccagagtc ttttagtcaa
361 ctggtttcgg atatgaagaa caaccactat gatgctaaaa catttgccct cgtatttcga
421 gctatggtag agaagtttga aagggattta agggaatcca aatttgcaga actcatgaac
481 aagcactttg ctgcaagttc aattccaaaa ggaattcact gtctctcttt aagactaacc
541 gatgaatatt cctccaatgc tcatgcccgg agacagcttc cttccccgga gcttctccct
601 gttctctcag acaatgctta ccaccatttt gttctagcta cagataatat cttagctgca
661 tcggttgtgg tctcatctgc tgttcaatca tcttcaaaac ccgagaaaat tgtcttccat
721 gttatcacag acaagaaaac ctatgcgggt atgcattctt ggtttgcact caattctgtt
781 gctcctgcga ttgttgaagt gaaaagcgtt catcagtttg attggttaac aagagagaat
841 gttccagttc ttgaagctgt ggaaagccat aacagtatca gaaattatta ccatgggaat
901 catattgctg gtgcaaacct cagcgaaaca acccctcgaa catttgcttc gaaactgcag
961 tcaagaagtc ccaaatacat atctttgctc aaccatctta gaatatatct accagagctt
1021 tttccgaact tagacaaggt agtgttctta gatgatgata tagtgataca gaaagattta
1081 tctccgcttt gggatattga ccttaacggg aaggttaatg gagctgtgga gacttgtcga
1141 ggagaagacg tatgggttat gtcaaagcgt cttaggaact acttcaattt ttctcacccg
1201 ctcatcgcaa agcatttaga tcccgaagaa tgtgcttggg cttatggaat gaatatcttt
1261 gatctacgga cttggaggaa gacaaatatc agagaaacgt atcattcttg gcttaaagag
1321 aatctgaagt cgaatctaac aatgtggaaa cttggaacat tgcctcctgc tctaatagca
1381 tttaaaggtc atgttcagcc aatagattcc tcttggcata tgcttggatt aggttatcag
1441 agcaagacca acttagaaaa tgcgaagaaa gctgcagtga ttcattacaa tggccaatca
1501 aagccgtggc ttgagatagg tttcgagcat ctcagaccat tctggacaaa atatgttaac
1561 tactccaatg atttcattaa gaattgtcat atcttggaat ag

Amino Acid Sequence of Sequence #9: (SEQ ID NO: 18)
Genebank ID# NP_186753
Positions 1-533 of NP_186753.

1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililafllp fvfiltavvt
61 legvnkcssf dcfgrrlgpr llgriddseq rlvrdfykil nevstqeipd glklpesfsq
121 lvsdmknnhy daktfalvfr amvekferdl reskfaelmn khfaassipk gihclslrlt
181 deyssnahar rqlpspellp vlsdnayhhf vlatdnilaa svvvssavqs sskpekivfh

241 vitdkktyag mhswfalnsv apaivevksv hqfdwltren vpvleavesh nsirnyyhgn
301 hiaganlset tprtfasklq srspkyisll nhlriylpel fpnldkvvfl dddiviqkdl
361 splwdidlng kvngavetcr gedvwvmskr lrnyfnfshp liakhldpee cawaygmnif
421 dlrtwrktni retyhswlke nlksnltmwk lgtlppalia fkghvqpids swhmlglgyq
481 sktnlenakk aavihyngqs kpwleigfeh lrpfwtkyvn ysndfiknch ile

Sequence #10 (SEQ ID NO: 19)

Gene name: At3g02350
GeneBank accession # for reference: NM_111102 GI:18396158
Nucleotide sequence of Sequence #10:
Positions 1-1686 of CDS of NM_111102.

1 atggcggtgg ccttccgtgg aggccgggga ggcgtcggat ccggccaatc taccggactt
61 cgtagtttct tctcctaccg gatctttatc tccgctttgt tctcttttct cttcctcgcc
121 actttctccg tcgttcttaa ctcctctcgt catcagcctc atcaggatca tacattgccg
181 agtatgggca acgcatatat gcagaggacg tttttggctt tgcaatcgga tccattgaaa
241 actaggttgg atctgataca caagcaagcc attgatcatt tgacactggt gaatgcgtat
301 gctgcttacg ctaggaagct aaagcttgat gcttctaagc agcttaagct cttcgaagat
361 ttggctatca acttctcgga tttgcagtcg aaacctggtt tgaaatctgc tgtgtctgat
421 aatggtaatg ctcttgagga ggattcgttt aggcagcttg agaaagaagt gaaggataag
481 gtgaagacag cgaggatgat gatcgttgag tctaaagaga gttatgatac acagcttaaa
541 atccagaagt tgaaagatac aatctttgct gtccaagaac agttgacaaa ggctaagaaa
601 aacggtgcgg ttgctagctt gatttcagcc aagtcggttc ctaaaagtct tcattgtttg
661 gccatgaggc ttgtaggaga gaggatctct aatcctgaga agtacaagga tgctccacct
721 gacccagccg cagaggatcc aactctttac cactatgcga ttttctctga taatgtcatt
781 gctgtgtctg ttgtggtgag atcggttgtg atgaacgctg aggagccatg gaagcatgtc
841 ttccatgtgg tgacagatcg gatgaatctc gcagccatga aggtgtggtt taagatgcgt
901 cctttggacc gtggtgccca tgttgagatt aaatccgtgg aggatttcaa gttcttaaac
961 tcttcctatg cgccggtctt gaggcagctt gagtctgcca agttgcagaa gttttacttt
1021 gagaatcaag ctgagaacgc aactaaagat tcacataacc tcaagttcaa gaaccccaag
1081 tatctctcga tgttgaacca tctcagattt tacttaccag agatgtatcc gaagctgaat
1141 aagatttttgt tcttggacga tgatgttgtg gtgcagaaag acgtgactgg tttatggaaa
1201 atcaacttgg atggcaaggt gaatggagcc gttgagacat gttttggttc ttttcatcga
1261 tatggtcaat acttaaactt ctctcatcct ttgatcaaag agaactttaa ccccagtgcc
1321 tgtgcttggg cctttggaat gaacatattc gatctcaatg cctggagacg cgagaagtgc
1381 accgatcaat accattactg gcagaacctg aatgaagaca gaactctctg gaaattggga
1441 actctacctc cgggattgat cacattctat tcaaagacga aatcattgga caaatcatgg
1501 catgtacttg ggttaggcta taacccggga gtgagcatgg acgaaatcag aaatgcagga
1561 gtgattcatt acaatggaaa catgaaaccg tggctagaca ttgcgatgaa ccaatacaag
1621 tctctctgga ctaaatatgt tgataacgaa atggagtttg tgcagatgtg caattttggt
1681 ctctaa

Amino Acid Sequence of Sequence #10: (SEQ ID NO: 20)
Genebank ID# NP_566170.1
Positions 1-561 of NP_566170.

1 mavafrggrg gvgsgqstgl rsffsyrifi salfsflfla tfsvvlnssr hqphqdhtlp
61 smgnaymqrt flalqsdplk trldlihkqa idhltlvnay aayarklkld askqlklfed
121 lainfsdlqs kpglksavsd ngnaleedsf rqlekevkdk vktarmmive skesydtqlk
181 iqklkdtifa vqeqltkakk ngavaslisa ksvpkslhcl amrlvgeris npekykdapp
241 dpaaedptly hyaifsdnvi avsvvvrsvv mnaeepwkhv fhvvtdrmnl aamkvwfkmr
301 pldrgahvei ksvedfkfln ssyapvlrql esaklqkfyf enqaenatkd shnlkfknpk
361 ylsmlnhlrf ylpemypkln kilfldddvv vqkdvtglwk inldgkvnga vetcfgsfhr
421 ygqylnfshp likenfnpsa cawafgmnif dlnawrrekc tdqyhywqnl nedrtlwklg
481 tlppglitfy sktksldksw hvlglgynpg vsmdeirnag vihyngnmkp wldiamnqyk
541 slwtkyvdne mefvqmcnfg l

Sequence #11 (SEQ ID NO: 21)

Gene name: at3g25140
GeneBank accession # for reference: NM_113418 GI:30687767
Nucleotide sequence of Sequence #11:
Positions 1-1680 of CDS of NM_113418.

1 atggctaatc accaccgact tttacgcggc ggcggatctc cggccataat cggtggcaga
61 atcacactca cagctttcgc ttccactatc gcactcttcc tcttcactct ctccttcttc
121 ttcgcttcag attctaacga ttctcctgat ctccttcttc ccggtgttga gtactctaat
181 ggagtcggat ctagaagatc catgttggat atcaaatcgg atccgcttaa gccacggttg
241 attcagatcc ggaaacaagc tgatgatcat cggtcattag cattagctta tgcttcttac
301 gcgagaaagc ttaagctcga gaattcgaaa ctcgtcagga tcttcgctga tctttcgagg
361 aattacacgg atctgattaa caaaccgacg tatcgagctt tgtatgattc tgatggagcc
421 tcgattgaag aatctgtgct taggcaattt gagaaagaag ttaaggaacg gattaaaatg
481 actcgtcaag tgattgctga agctaaagag tcttttgata atcagttgaa gattcagaag
541 ctgaaagata cgattttcgc tgttaacgaa cagttaacta atgctaagaa gcaaggtgcg
601 ttttcgagtt tgatcgctgc gaaatcgatt ccgaaaggat tgcattgtct tgctatgagg
661 ctgatggaag agaggattgc tcaccctgag aagtatactg atgaagggaa agatagaccg
721 cgggagctcg aggatccgaa tctttaccat tacgctatat tttcggataa tgtgattgcg
781 gcttcggtgg ttgtgaactc tgctgtgaag aatgctaagg agccgtggaa gcatgttttt
841 cacgttgtga ctgataagat gaatcttgga gctatgcagg ttatgtttaa actgaaggag
901 tataaaggag ctcatgtaga agttaaagct gttgaggatt atacgttttt gaactcttcg
961 tatgtgcctg tgttgaagca gttagaatct gcgaatcttc agaagtttta tttcgagaat
1021 aagctcgaga atgcgacgaa agataccacg aatatgaagt tcaggaaccc caagtattta
1081 tctatattga atcacttgag gttttattta cccgagatgt acccgaaact acataggata
1141 ctgtttttgg acgatgatgt ggttgtgcag aaggatttaa cgggtctgtg ggagattgat
1201 atggatggga aagtgaatgg agctgtagag acttgttttg ggtcgtttca tcggtacgct
1261 caatacatga atttctcaca tcctttgatc aaagagaagt ttaatcccaa agcatgtgcg
1321 tgggcgtatg gaatgaactt ctttgatctt gatgcttgga gaagagagaa gtgcacagaa
1381 gaatatcact actggcaaaa tctgaacgag aacagggctc tatggaaact ggggacgtta
1441 ccaccgggac tgatcacctt ttactcaacc acaaagccgc tggacaaatc atggcatgtg
1501 cttgggctgg gttacaatcc gagcattagc atggatgaga tccgcaacgc tgcagtggta
1561 cacttcaacg gtaacatgaa gccatggctt gacatagcta tgaaccagtt tcgaccactt
1621 tggaccaaac acgtcgacta tgacctcgag tttgttcagg cttgcaattt tggcctctga

Amino Acid Sequence of Sequence #11: (SEQ ID NO: 22)
Genebank ID# NP_189150
Positions 1-559 of NP_189150.

1 manhhrllrg ggspaiiggr itltafasti alflftlsff fasdsndspd lllpgveysn
61 gvgsrrsmld iksdplkprl iqirkqaddh rslalayasy arklklensk lvrifadlsr
121 nytdlinkpt yralydsdga sieesvlrqf ekevkerikm trqviaeake sfdnqlkiqk
181 lkdtifavne qltnakkqga fssliaaksi pkglhclamr lmeeriahpe kytdegkdrp
241 reledpnlyh yaifsdnvia asvvvnsavk nakepwkhvf hvvtdkmnlg amqvmfklke
301 ykgahvevka vedytflnss yvpvlkqles anlqkfyfen klenatkdtt nmkfrnpkyl
361 silnhlrfyl pemypklhri lfldddvvvq kdltglweid mdgkvngave tcfgsfhrya
421 qymnfshpli kekfnpkaca waygmnffdl dawrrekcte eyhywqnlne nralwklgtl
481 ppglitfyst tkpldkswhv lglgynpsis mdeirnaavv hfngnmkpwl diamnqfrpl
541 wtkhvdydle fvqacnfgl

Sequence #12 (SEQ ID NO: 23)

Gene name: At3g58790
GeneBank accession # for reference: NM_115741 GI:22331856
Nucleotide sequence of Sequence #12:
Positions 1-1623 of CDS of NM_115741.

1 atgaagtttt acatatcagc gacggggatt aagaaggtta cgatatcaaa tcccggcgtc
61 ggaatcggta aaggaagcgg aggatgtgcg gctgcagcgg cggcgttagc agcgcggaga
121 ttctctagtc gcacgttgtt actgttgctg ctgctgctcg ctatcgtcct ccctttttatc
181 ttcgtcaggt tcgcgtttct cgtcctcgaa tctgcctccg tttgcgattc accactcgat
241 tgcatgggac tcagactttt ccgtgggggc gacacatctc tgaaaattgg ggaagagttg
301 acacgggctc tagtggaaga gacgacagat catcaggacg ttaatggaag aggaacgaag
361 ggatcattgg agtcattcga cgaccttgtt aaggagatga cgttaaaacg ccgtgacata
421 agggcgtttg cttccgtgac taagaagatg ctgttgcaga tggaacgtaa agtccaatca
481 gcgaaacatc atgagttagt gtactggcat ttagcctctc acggtattcc taaaagcctc
541 cattgccttt ccctcagatt aactgaagag tactctgtaa atgcaatggc tcgaatgcgt
601 ttgcctccgc ctgagtccgt atcacgtctg accgacccat cttttcatca tattgtcctc
661 ctgactgaca atgtccttgc tgcctctgtc gtcatatcgt ctactgtaca aaacgctgtg
721 aatcccgaga agtttgtctt tcatattgtt accgataaga aaacctatac ccctatgcat
781 gcttggtttg ctatcaactc tgcttcatca ccagttgttg aagtaaaggg acttcatcag
841 tatgattggc ctcaagaagt gaacttcaaa gttagagaga tgctggacat tcaccgctta
901 atttggagac gacattatca aaatttgaaa gactctgatt ttagttttgt tgagggtact
961 catgagcagt ccttgcaagc tctaaatcct agctgccttg ccctttttgaa ccatcttcgc
1021 atttacattc ccaagctttt tccagatctc aacaagatag tgttgttgga tgatgatgta
1081 gtagtacaga gcgatctttc gtctttatgg gaaacggatc tcaacggtaa agttgttggt
1141 gctgtcgttg attcgtggtg cggagacaac tgttgccccg gaagaaaata caaagactat
1201 ttcaacttct cacatccttt gatctcatca aacttagttc aagaagactg tgcttggctt
1261 tctggtatga atgtctttga tctcaaagcc tggagacaaa ccaatattac tgaagcttac
1321 tctacatggc taagactcag tgttaggtca ggactacaat tatggcaacc aggggcttta
1381 ccaccgacat tacttgcttt caaaggactt acacagtctc ttgaaccatc atggcacgtc
1441 gctggactag gttctcgatc cgtaaaatcc cctcaagaga ttctgaaatc tgcttcggtt
1501 ttacatttca gcggtccagc aaaaccgtgg ctagagatca gtaaccctga ggtacgatct
1561 ctttggtata gatacgtaaa ttcctccgac atcttcgtta gaaaatgcaa aatcatgaac
1621 tga

Amino Acid Sequence of Sequence #12: (SEQ ID NO: 24)
Genebank ID# NP_191438.2
Positions 1-540 of NP_191438.

1 mkfyisatgi kkvtisnpgv gigkgsggca aaaaalaarr fssrtllll lllaivlpfi
61 fvrfaflvle sasvcdspld cmglrlfrgg dtslkigeel tralveettd hqdvngrgtk
121 gslesfddlv kemtlkrrdi rafasvtkkm llqmerkvqs akhhelvywh lashgipksl
181 hclslrltee ysvnamarmr lpppesvsrl tdpsfhhivl ltdnvlaasv visstvqnav
241 npekfvfhiv tdkktytpmh awfainsass pvvevkglhq ydwpqevnfk vremldihrl
301 iwrrhyqnlk dsdfsfvegt heqslqalnp sclallnhlr iyipklfpdl nkivlldddv
361 vvqsdlsslw etdlngkvvg avvdswcgdn ccpgrkykdy fnfshpliss nlvqedcawl
421 sgmnvfdlka wrqtniteay stwlrlsvrs glqlwqpgal pptllafkgl tqslepswhv
481 aglgsrsvks pqeilksasv lhfsgpakpw leisnpevrs lwyryvnssd ifvrkckimn

Sequence #13 (SEQ ID NO: 25)

Gene name: At4g38270
GeneBank accession # for reference: NM_119989 GI: 30691874
Nucleotide sequence of Sequence #13
Positions 1-2043 of CDS of NM_119989.

1 atgacgacgt tctctacatg cgccgccttt ttatcgctgg tagtagtgct acatgctgtt
61 catgtcggtg gagccatttt agagtcacaa gcaccccaca gagaacttaa agcttatcgt
121 ccgctgcaag ataataatct acaggaggtg tatgcttcct cagctgctgc agtgcactac
181 gatccagatc tgaaagatgt gaacatagtt gcgacataca gtgaccatta cggcaatata
241 cgccttggta gggtgaaaat gggggatctt tcaccttctt gggttttgga gaatcctgcc
301 tatcaagtta gccgcaaaac aaaaggttcg cagctagtta taccacggga ttcatttcaa
361 aatgatactg gaatggaaga taatgcaagc cattctacaa ctaatcagac tgatgaaagc
421 gaaaatcagt ttccaaacgt ggattttgca agcccagcaa aactgaagcg gcagatttta
481 cgtcaggaaa ggagaggtca acgaacttta gagctgatcc gacaagaaaa ggaaactgat
541 gagcagatgc aagaagcagc cattcagaag tcaatgagct ttgaaaactc agtcataggg
601 aaatacagta tatggaggag agactatgag agcccaaatg ctgatgctat cttgaagctt
661 atgagagacc agatcataat ggcaaaagca tatgcaaata ttgccaaatc aaaaaatgta
721 accaatctgt acgttttctt gatgcagcag tgtggagaaa ataaacgtgt tataggtaaa
781 gcaacctctg atgctgacct tccttcaagc gctcttgatc aagcaaaagc catgggccat
841 gcactctctc ttgcaaaaga cgagttatat gactgccatg aacttgcaaa aaagttccgg
901 gccatccttc agtccactga acgcaaagta gatggactga agaaaaaggg aaccttctta
961 attcagctag ctgccaaaac atttcccaag ccattgcatt gcctgagtct gcagctagcg
1021 gcagactatt ttattctagg tttcaatgaa gaggatgcag tgaaagagga tgtcagtcaa
1081 aagaagcttg aagatccttc gctctatcac tatgcgatct tttcggataa cgttctggct
1141 acatcagtgg tggtgaactc cactgtcttg aatgcaaagg aaccgcagag gcatgtgttc
1201 catatagtaa ctgacaaact gaattttggt gcaatgaaga tgtggtttcg catcaatgct
1261 cctgctgatg cgacgattca agttgaaaac ataaatgatt tcaagtggct gaactcctct
1321 tactgctctg ttctacggca gcttgaatct gcaaggctga aagaatacta tttcaaagca
1381 aatcatcctt catcaatctc agctggcgca gataatctaa agtaccgcaa cccaaagtat
1441 ctatcgatgc tgaatcatct cagattctac cttcctgagg tttatccgaa gctggagaag
1501 attctgtttc tagacgatga cattgtggtg cagaaggacc tggcaccact atgggaaata
1561 gacatgcaag gaaaagtgaa tggtgcggtg gagacgtgca aggagagctt ccacagattt
1621 gacaagtacc tcaacttctc aaatccaaag atttcagaga attttgacgc tggtgcttgt
1681 gggtgggcat ttgggatgaa tatgtttgac ctgaaagagt ggaggaaacg gaacattaca

1741 gggatatatc actattggca agacttgaat gaagacagaa cactgtggaa gctgggatcg
1801 ttgccaccgg ggctgataac attttacaac ctgacgtatg caatggatag gagctggcac
1861 gtactagggc tgggatatga cccagcgcta aaccaaacag caatagagaa tgcagcggta
1921 gtgcattaca atgggaacta caagccatgg ctgggtttag cattcgccaa gtacaaaccg
1981 tactggtcca agtacgttga gtacgacaac ccttatctcc gacggtgcga catcaatgaa
2041 tga

Amino Acid Sequence of Sequence #13: (SEQ ID NO: 26)
Genebank ID# NP_195540.2
Positions 1-680 of NP_195540.

1 mttfstcaaf lslvvvlhav hvggailesq aphrelkayr plqdnnlqev yassaaavhy
61 dpdlkdvniv atysdhygni rlgrvkmgdl spswvlenpa yqvsrktkgs qlviprdsfq
121 ndtgmednas hsttnqtdes enqfpnvdfa spaklkrqil rqerrgqrtl elirqeketd
181 eqmqeaaiqk smsfensvig kysiwrrdye spnadailkl mrdqiimaka yaniaksknv
241 tnlyvflmqq cgenkrvigk atsdadlpss aldqakamgh alslakdely dchelakkfr
301 ailqsterkv dglkkkgtfl iqlaaktfpk plhclslqla adyfilgfne edavkedvsq
361 kkledpslyh yaifsdnvla tsvvvnstvl nakepqrhvf hivtdklnfg amkmwfrina
421 padatiqven indfkwlnss ycsvlrqles arlkeyyfka nhpssisaga dnlkyrnpky
481 lsmlnhlrfy lpevypklek ilfldddivv qkdlaplwei dmqgkvngav etckesfhrf
541 dkylnfsnpk isenfdagac gwafgmnmfd lkewrkrnit giyhywqdln edrtlwklgs
601 lppglitfyn ltyamdrswh vlglgydpal nqtaienaav vhyngnykpw lglafakykp
661 ywskyveydn pylrrcdine

Sequence #14 (SEQ ID NO: 27)

Gene name: At5g15470
GeneBank accession # for reference: NM_121551 GI:30685368
Nucleotide sequence of Sequence #14:
Positions 1-1599 of CDS of NM_121551.

1 atgcagcttc acatatcgcc gagtatgaga agcattacga tttcgagcag caatgagttt
61 attgacttga tgaagatcaa ggtcgcagct cgtcacatct cttaccgaac tctcttccac
121 accatcttaa tcctcgcttt cttgttgcct tttgttttca ttctcaccgc tgttgttacc
181 cttgagggtg tcaacaaatg ctcctccatt gattgtttag ggaggcggat aggtccacgt
241 cttcttggta gggtagatga ttcagagaga ctagctagag acttttataa aattctaaac
301 gaagtaagca ctcaagaaat tccagatggt ttgaagcttc caaattcttt tagtcaactt
361 gtttccgata tgaagaataa ccactatgat gcaaaaacat ttgctcttgt gctgcgagcc
421 atgatggaga agtttgaacg tgatatgagg gaatcgaaat ttgcagaact tatgaacaag
481 cactttgcag caagttccat tcccaaaggc attcattgtc tctctctaag actgacagat
541 gaatattcct ccaatgctca tgctcgaaga cagcttcctt caccagagtt tctccctgtt
601 ctttcagata atgcttacca ccactttatt ttgtccacgg acaatatttt ggctgcctca
661 gttgtggtct catccgctgt tcagtcatct tcaaaacccg agaaaattgt ctttcacatc
721 attacagaca agaaaaccta tgcgggtatg cattcatggt ttgcgcttaa ttctgttgca
781 ccagcaattg ttgaggttaa aggtgttcat cagtttgact ggttgacgag agagaatgtt
841 ccggttttgg aagctgtgga aagccataat ggtgtcaggg actattatca tgggaatcat
901 gtcgctgggg caaacctcac cgaaacaact cctcgaacat ttgcttcaaa attgcagtct
961 agaagtccaa aatacatatc tttgctcaac catcttagaa tatatatacc agagcttttc
1021 ccgaacttgg acaaggtggt tttcttagac gatgatatag ttgtccaggg agacttaact

1081 ccactttggg atgttgacct cggtggtaag gtcaatgggg cagtagagac ttgcaggggt
1141 gaagatgaat gggtgatgtc aaagcgttta aggaactact tcaatttctc tcacccgctc
1201 atcgcaaagc atttagatcc tgaagaatgt gcttgggcat atggtatgaa tatcttcgat
1261 ctacaagctt ggaggaaaac aaatatcaga gaaacgtatc actcttggct tagagagaat
1321 ctaaagtcaa atctgacaat gtggaaactt ggaaccttgc ctcctgctct tatcgcgttc
1381 aagggtcacg tacacataat agactcgtca tggcatatgc taggattagg ctaccagagc
1441 aagaccaaca tagaaaatgt gaagaaagca gcagtgatcc actacaatgg gcagtcaaag
1501 ccatggctgg agattggttt cgagcatctg cggccattct ggaccaaata cgtcaactac
1561 tcaaatgatt tcatcaagaa ctgtcacata ttggagtag

Amino Acid Sequence of Sequence #14: (SEQ ID NO: 28)
Genebank ID# NP_197051
Positions 1-532 of NP_197051.

1 mqlhispsmr sitisssnef idlmkikvaa rhisyrtlfh tililaflip fvfiltavvt
61 legvnkcssi dclgrrigpr llgrvddser lardfykiln evstqeipdg lklpnsfsql
121 vsdmknnhyd aktfalvlra mmekferdmr eskfaelmnk hfaassipkg ihclslrltd
181 eyssnaharr qlpspeflpv lsdnayhhfi lstdnilaas vvvssavqss skpekivfhi
241 itdkktyagm hswfalnsva paivevkgvh qfdwltrenv pvleaveshn gvrdyyhgnh
301 vaganltett prtfasklqs rspkyislln hlriyipelf pnldkvvfld ddivvqgdlt
361 plwdvdlggk vngavetcrg edewvmskrl rnyfnfshpl iakhldpeec awaygmnifd
421 lqawrktnir etyhswlren lksnltmwkl gtlppaliaf kghvhiidss whmlglgyqs
481 ktnienvkka avihyngqsk pwleigfehl rpfwtkyvny sndfiknchi le

Sequence #15 (SEQ ID NO: 29)

Gene name: At5g54690
GeneBank accession # for reference: NM_124850 GI:30696504
Nucleotide sequence of Sequence #15:
Positions 1-1608 of CDS of NM_124850.

1 atgcagttac atatatctcc gagcttgaga catgtgactg tggtcacagg gaaaggattg
61 agagagttca taaaagttaa ggttggttct agaagattct cttatcaaat ggtgttttac
121 tctctactct tcttcacttt tcttctccga ttcgtctttg ttctctccac cgttgatact
181 atcgacggcg atccctctcc ttgctcctct cttgcttgct tggggaaaag actaaagcca
241 aagcttttag gaagaagggt tgattctggt aatgttccag aagctatgta ccaagtttta
301 gaacagcctt taagcgaaca agaactcaaa ggaagatcag atatacctca aacacttcaa
361 gatttcatgt ctgaagtcaa aagaagcaaa tcagacgcaa gagaatttgc tcaaaagcta
421 aaagaaatgg tgacattgat ggaacagaga acaagaacgg ctaagattca agagtattta
481 tatcgacatg tcgcatcaag cagcataccg aaacaacttc actgtttagc tcttaaacta
541 gccaacgaac actcgataaa cgcagcggcg cgtctccagc ttccagaagc tgagcttgtc
601 cctatgttgg tagacaacaa ctactttcac tttgtcttgg cttcagacaa tattcttgca
661 gcttcggttg tggctaagtc gttggttcaa aatgctttaa gacctcataa gatcgttctt
721 cacatcataa cggataggaa aacttatttc ccaatgcaag cttggttctc attgcatcct
781 ctgtctccag caataattga ggtcaaggct ttgcatcatt tcgattggtt atcgaaaggt
841 aaagtacccg ttttggaagc tatggagaaa gatcagagag tgaggtctca attcagaggt
901 ggatcatcgg ttattgtggc taataacaaa gagaacccgg ttgttgttgc tgctaagtta
961 caagctctca gccctaaata caactccttg atgaatcaca tccgtattca tctaccagag
1021 ttgtttccaa gcttaaacaa ggttgtgttt ctagacgatg acattgtgat ccaaactgat

1081 ctttcacctc tttgggacat tgacatgaat ggaaaagtaa atggagcagt ggaaacatgt
1141 agaggagaag acaagtttgt gatgtcaaag aagttcaaga gttacctcaa cttctcgaat
1201 ccgacaattg ccaaaaactt caatccagag gaatgtgcat gggcttatgg aatgaatgtt
1261 ttcgacctag cggcttggag gaggactaac ataagctcca cttactatca ttggcttgac
1321 gagaacttaa aatcagacct gagtttgtgg cagctgggaa ctttgcctcc tgggctgatt
1381 gctttccacg gtcatgtcca aaccatagat ccgttctggc atatgcttgg tctcggatac
1441 caagagacca cgagctatgc cgatgctgaa agtgccgctg ttgttcattt caatggaaga
1501 gctaagcctt ggctggatat agcatttcct catctacgtc ctctctgggc taagtatctt
1561 gattcttctg acagatttat caagagctgt cacattagag catcatga

Amino Acid Sequence of Sequence #15: (SEQ ID NO: 30)
Genebank ID# NP_200280
Positions 1-535 of NM_200280.

1 mqlhispslr hvtvvtgkgl refikvkvgs rrfsyqmvfy sllfftfllr fvfvlstvdt
61 idgdpspcss laclgkrlkp kllgrrvdsg nvpeamyqvl eqplseqelk grsdipqtlq
121 dfmsevkrsk sdarefaqkl kemvtlmeqr trtakiqeyl yrhvasssip kqlhclalkl
181 anehsinaaa rlqlpeaelv pmlvdnnyfh fvlasdnila asvvakslvq nalrphkivl
241 hiitdrktyf pmqawfslhp lspaiievka lhhfdwlskg kvpvleamek dqrvrsqfrg
301 gssvivannk enpvvvaakl qalspkynsl mnhirihlpe lfpslnkvvf ldddiviqtd
361 lsplwdidmn gkvngavetc rgedkfvmsk kfksylnfsn ptiaknfnpe ecawaygmnv
421 fdlaawrrtn isstyyhwld enlksdlslw qlgtlppgli afhghvqtid pfwhmlglgy
481 qettsyadae saavvhfngr akpwldiafp hlrplwakyl dssdrfiksc hiras

The nucleotide and amino acid sequences of the ten *GALAT-LIKE* gene family members are shown as follows.

Sequence #16 (SEQ ID NO:31)

Gene name: At1g02720
GeneBank accession # for reference: NM_100152, GI: 30678358
Nucleotide sequence of Sequence #16:
Positions 1-1086 of CDS of NM_100152.

1 atgcattgga ttacgagatt ctctgctttc ttctccgccg cattagccat gattctcctt
61 tctccttcgc tccaatcctt ttctccggcg gcagctatcc gatcatctca cccctacgcc
121 gacgaattca aaccccaaca aaactccgat tactcctcct tcagagaatc tccaatgttc
181 cgtaacgccg aacaatgcag atcttccggc gaagattccg gcgtctgtaa ccctaatctc
241 gtccacgtag ccatcactct cgacatcgat tacctccgtg gctcaatcgc agccgtcaat
301 tcgatcctcc agcactcaat gtgccctcaa agcgtcttct tccacttcct cgtctcctcc
361 gagtctcaaa acctagaatc tctgattcgt tctactttcc ccaaattgac gaatctcaaa
421 atttactatt ttgcccctga gaccgtacag tctttgattt catcttccgt gagacaagcc
481 ctagagcaac cgttgaatta cgccagaaat tacttggcgg atctgctcga gccttgcgtt
541 aagcgagtca tctacttgga ttcggatctc gtcgtcgtcg atgatatcgt caagctttgg
601 aaaacgggtt taggccagag aacaatcgga gctccggagt attgtcacgc gaatttcacg
661 aaatacttca ccggaggttt ttggtcagat aagaggttta acgggacgtt caaagggagg
721 aacccttgtt acttcaatac tggtgtaatg gtgattgatt tgaagaagtg gagacaattt

781 aggttcacga aacgaattga gaaatggatg gagattcaga agatagagag gatttatgag
841 cttggttctc ttcctccgtt tcttctggta tttgctggtc atgtagctcc gatttcacat
901 cggtggaatc aacatgggct tggtggtgat aatgttagag gtagttgccg tgatttgcat
961 tctggtcctg tgagtttgct tcactggtca ggtagtggta agccatggtt aagactcgat
1021 tccaagcttc catgtccttt agacacattg tgggcacctt atgatttgta taaacactcc
1081 cattga

Amino Acid Sequence of Sequence #16: (SEQ ID NO: 32)
Genebank ID# NP_171772
Positions 1-361.

1 mhwitrfsaf fsaalamill spslqsfspa aairsshpya defkpqqnsd yssfrespmf
61 rnaeqcrssg edsgvcnpnl vhvaitldid ylrgsiaavn silqhsmcpq svffhflvss
121 esqnleslir stfpkltnlk iyyfapetvq slisssvrqa leqplnyarn yladllepcv
181 krviyldsdl vvvddivklw ktglgqrtig apeychanft kyftggfwsd krfngtfkgr
241 npcyfntgvm vidlkkwrqf rftkriekwm eiqkieriye lgslppfllv faghvapish
301 rwnqhglggd nvrgscrdlh sgpvsllhws gsgkpwlrld sklpcpldtl wapydlykhs
361 h

Sequence #17 (SEQ ID NO:33)

Gene name: At1g13250
GeneBank accession # for reference: NM_101196, GI:30683194
Nucleotide sequence of Sequence #17:
Positions 1-1038 of CDS of NM_101196.

1 atgtcttctc tgcgtttgcg tttatgtctt cttctactct tacctatcac aattagctgc
61 gtcacagtca ctctcactga cctccccgcg tttcgtgaag ctccggcgtt tcgaaacggc
121 agagaatgct ccaaaacgac atggatacct tcggatcacg aacacaaccc atcaatcatc
181 cacatcgcta tgactctcga cgcaatttac ctccgtggct cagtcgccgg cgtcttctcc
241 gttctccaac acgcttcttg tcctgaaaac atcgttttcc acttcatcgc cactcaccgt
301 cgcagcgccg atctccgccg cataatctcc tcaacattcc catacctaac ctaccacatt
361 taccattttg accctaacct cgtccgcagc aaaatatctt cctctattcg tcgtgcttta
421 gaccaaccgt taaactacgc tcggatctac ctcgccgatc tcctcccaat cgccgtccgc
481 cgcgtaatct acttcgactc cgatctcgta gtcgtcgatg acgtggctaa actctggaga
541 atcgatctac gtcggcacgt cgtcggagct ccggagtact gtcacgcgaa tttcactaac
601 tacttcactt caagattctg gtcgagtcaa ggttacaaat cggcgttgaa agataggaaa
661 ccgtgttatt tcaacaccgg agtgatggtg attgatctcg gaaaatggag agaaaggaga
721 gtcacggtga agctagagac atggatgagg attcaaaaac gacatcgtat ttacgaattg
781 ggatctttgc ctccgtttct gctcgttttc gccggagatg ttgagccggt ggagcatagg
841 tggaatcagc atggtcttgg tggtgataac ttggaaggac tttgccggaa tttgcatcca
901 ggtccggtga gtttgttgca ttggagcggg aaagggaaac catggctaag gcttgactcg
961 agacgaccgt gtccgttgga ttcgttatgg gctccttatg atttgtttcg ttattcaccg
1021 ttgatctctg atagctga

Amino Acid Sequence of Sequence #17: (SEQ ID NO: 34)
Genebank ID# NP_563925
Positions 1-345.

1 msslrlrlcl lllpitisc vtvtltdlpa freapafrng recskttwip sdhehnpsii
61 hiamtldaiy lrgsvagvfs vlqhascpen ivfhfiathr rsadlrriis stfpyltyhi
121 yhfdpnlvrs kisssirral dqplnyariy ladllpiavr rviyfdsdlv vvddvaklwr
181 idlrrhvvga peychanftn yftsrfwssq gyksalkdrk pcyfntgvmv idlgkwrerr
241 vtvkletwmr iqkrhriyel gslppfllvf agdvepvehr wnqhglggdn leglcrnlhp
301 gpvsllhwsg kgkpwlrlds rrpcpldslw apydlfrysp lisds

Sequence #18 (SEQ ID NO:35)

Gene name: At1g19300
GeneBank accession # for reference: NM_101787, GI:30686302
Nucleotide sequence of Sequence #18:
Positions 1-1056 of CDS of NM_101787.

1 atgtcccaac atcttcttct tctcattctc ctctcgctac ttcttcttca taaacccatt
61 tccgccacta caattattca aaaattcaaa gaagccccac agttttacaa ttctgcagat
121 tgccccttaa tcgatgactc cgagtccgac gatgacgtgg tcgccaaacc aatcttctgc
181 tcacgtcgag ctgtccacgt ggcgatgaca ctcgacgccg cctacattcg tggctcagtc
241 gccgctgttc tctccgtcct ccaacactct tcttgtcctg aaaacattgt tttccacttc
301 gtcgcctctg cttccgccga cgcttcttcc ttacgagcca ccatatcctc ctctttccct
361 taccttgatt tcaccgtcta cgtcttcaac gtctcctccg tctctcgcct tatctcctcc
421 tctatccgct ccgcactaga ctgtccttta aactacgcaa gaagctacct cgccgatctc
481 ctccctccct gcgtccgccg cgtcgtctac ctagactccg atctgatcct cgtcgacgac
541 atagcaaaac tcgccgccac agatctcggc cgtgattcag tcctcgccgc gccggaatac
601 tgcaacgcca atttcacttc atacttcaca tcaaccttct ggtctaatcc gactctctct
661 ttaaccttcg ccgatcggaa agcatgctac ttcaacactg gagtcatggt gatcgatctt
721 tcccggtggc gcgaaggcgc gtacacgtca cgcatcgaag agtggatggc gatgcaaaag
781 agaatgagaa tttacgagct tggttcgtta ccaccgtttt tattggtttt tgccggtttg
841 attaaaccgg ttaatcatcg gtggaaccaa cacggtttag gaggtgataa tttcagagga
901 ctgtgtagag atctccatcc tggtccggtg agtctgttgc attggagtgg gaaaggtaag
961 ccatgggcta ggcttgatgc tggtcggcct tgtcctttag acgcgctttg ggctccgtat
1021 gatcttcttc aaacgccgtt cgcgttggat tcttga

Amino Acid Sequence of Sequence #18: (SEQ ID NO: 36)
Genebank ID# NP_564077
Positions 1-351.

1 msqhllllil lsllllhkpi sattiiqkfk eapqfynsad cpliddsesd ddvvakpifc
61 srravhvamt ldaayirgsv aavlsvlqhs scpenivfhf vasasadass lratisssfp
121 yldftvyvfn vssvsrliss sirsaldcpl nyarsyladl lppcvrrvvy ldsdlilvdd
181 iaklaatdlg rdsvlaapey cnanftsyft stfwsnptls ltfadrkacy fntgvmvidl
241 srwregayts rieewmamqk rmriyelgsl ppfllvfagl ikpvnhrwnq hglggdnfrg
301 lcrdlhpgpv sllhwsgkgk pwarldagrp cpldalwapy dllqtpfald s

Sequence #19 (SEQ ID NO:37)

Gene name: At1g24170
GeneBank accession # for reference: NM_102263, GI:30688765
Nucleotide sequence of Sequence #19:
Positions 1-1182 of CDS of NM_102263.

```
   1 atgtcgtcgc gtttttcttt gacggtggtg tgtttgattg ctctgttacc gtttgttgtt
  61 ggtatacggt tgattccggc gaggatcacg agtgtcggtg atggcggcgg cggaggaggt
 121 aataatgggt ttagtaaact tggtccgttt atggaagctc cggagtatag aaacggcaag
 181 gagtgtgtat cttcatcagt gaacagagag aacttcgtgt cgtcttcttc tagttctaat
 241 gatccttcgc ttgttcacat cgctatgact ttggactcag agtatctccg tggatcaatc
 301 gcagccgttc attctgttct tcgccacgcg tcttgtccag agaacgtctt cttccatttc
 361 atcgctgctg agtttgactc tgcgagtcct cgtgttctga gtcaactcgt gaggtcgact
 421 tttccttcgt tgaactttaa agtctacatt tttagggaag atacggtgat caatctcata
 481 tcttcttcga ttagactagc tttggagaat ccgttgaact atgctcggaa ctatctcgga
 541 gatattcttg atcgaagtgt tgaacgagtc atttatcttg actcggatgt tataactgtg
 601 gatgatatca caaagctttg gaacacggtt ttgaccgggt cacgagtcat cggagctccg
 661 gagtattgtc acgcgaactt cactcagtat ttcacttccg ggttctggtc agacccggct
 721 ttaccgggtc taatctcggg tcaaaagcct tgctatttca acacaggagt gatggtgatg
 781 gatcttgtta gatggagaga agggaattac agagagaagt tagagcaatg gatgcaattg
 841 cagaagaaga tgagaatcta cgatcttgga tcattaccac cgtttctttt ggtgtttgcg
 901 ggtaatgttg aagctattga tcatagatgg aaccaacatg gtttaggagg agacaatata
 961 cgaggaagtt gtcggtcatt gcatcctggt cctgtgagct tgttgcattg gagtggtaaa
1021 ggtaagccat gggttagact tgatgagaag aggccttgtc cgttggatca tctttgggag
1081 ccatatgatt tgtataagca taagattgag agagctaaag atcagtctct gcttgggttt
1141 gcttctctgt cggagttgac tgatgattca agcttcttgt ga
```

Amino Acid Sequence of Sequence #19: (SEQ ID NO: 38)
Genebank ID# NP_173827
Positions 1-393.

```
   1 mssrfsltvv cliallpfvv girliparit svgdgggggg nngfsklgpf meapeyrngk
  61 ecvsssvnre nfvssssssn dpslvhiamt ldseylrgsi aavhsvlrha scpenvffhf
 121 iaaefdsasp rvlsqlvrst fpslnfkvyi fredtvinli sssirlalen plnyarnylg
 181 dildrsverv iyldsdvitv dditklwntv ltgsrvigap eychanftqy ftsgfwsdpa
 241 lpglisgqkp cyfntgvmvm dlvrwregny rekleqwmql qkkmriydlg slppfllvfa
 301 gnveaidhrw nqhglggdni rgscrslhpg pvsllhwsgk gkpwvrldek rpcpldhlwe
 361 pydlykhkie rakdqsllgf aslseltdds sfl
```

Sequence #20 (SEQ ID NO:39)

Gene name: At1g70090
GeneBank accession # for reference: NM_105677, GI:30697975
Nucleotide sequence of Sequence #20:
Positions 1-1173 of CDS of NM_105677.

```
   1 atgcggttgc gttttccgat gaaatctgcc gttttagcgt ttgctatctt tctggtgttt
  61 attccactgt tttccgtcgg tatacggatg attccgggaa gactcaccgc cgtatccgcc
 121 accgtcggaa atggctttga tctggggtcg ttcgtggaag ctccggagta cagaaacggc
 181 aaggagtgcg tgtctcaatc gttgaacaga gaaaacttcg tgtcgtcttg cgacgcttcg
 241 ttagttcatg tagctatgac gcttgactcg gagtacttac gtggctcaat cgcagccgta
 301 cattcaatgc tccgccacgc gtcgtgtcca gaaaacgtct tcttccatct catcgctgca
 361 gagtttgacc cggcgagtcc acgcgttctg agtcaactcg tccgatctac tttcccgtcg
 421 ctaaacttca aagtctacat tttccgggaa gatacggtga tcaaccttat ctcttcttca
 481 atcagacaag ctttagagaa tccattgaac tatgctcgga actacctcgg agatattctt
 541 gatccatgcg tagacagagt catttaccta gactcggaca tcatcgtcgt cgatgacata
 601 acaaagcttt ggaacacgag tttgacaggg tcaagaatca tcggagctcc ggagtattgt
 661 cacgctaact tcacaaagta cttcacttca ggtttctggt ccgacccggc tttacccggt
 721 ttcttctcgg gtcgaaagcc ttgttatttc aacacgggtg tgatggtgat ggatctagtt
 781 agatggagag aaggaaacta cagagaaaag cttgaaactt ggatgcagat acagaagaag
 841 aagagaatct acgatttggg ttctttgcct ccgtttcttc ttgtcttcgc agggaacgtt
 901 gaagcaattg atcataggtg gaaccaacat ggtttaggag gagacaatgt acgaggaagt
 961 tgtaggtctt tgcataaagg accagtgagt ttgttgcatt ggagtggtaa aggtaagcca
1021 tgggtgagac ttgatgagaa gagaccgtgt ccgttggatc atttatggga accgtatgat
1081 ttatatgagc ataagattga aagagctaaa gatcagtctt tgttcgggtt ctcttctttg
1141 tctgagttaa cagaagattc aagctttttc tga
```

Amino Acid Sequence of Sequence #20: (SEQ ID NO: 40)
Genebank ID# NP_564983
Positions 1-390.

```
   1 mrlrfpmksa vlafaiflvf iplfsvgirm ipgrltavsa tvgngfdlgs fveapeyrng
  61 kecvsqslnr enfvsscdas lvhvamtlds eylrgsiaav hsmlrhascp envffhliaa
 121 efdpasprvl sqlvrstfps lnfkvyifre dtvinlisss irqalenpln yarnylgdil
 181 dpcvdrviyl dsdiivvddi tklwntsltg sriigapeyc hanftkyfts gfwsdpalpg
 241 ffsgrkpcyf ntgvmvmdlv rwregnyrek letwmqiqkk kriydlgslp pfllvfagnv
 301 eaidhrwnqh glggdnvrgs crslhkgpvs llhwsgkgkp wvrldekrpc pldhlwepyd
 361 lyehkierak dqslfgfssl seltedssff
```

Sequence #21 (SEQ ID NO:41)

Gene name: At3g06260
GeneBank accession # for reference: NM_111501, GI:18397517
Nucleotide sequence of Sequence #21:
Positions 1-1056 of CDS of NM_111501.

```
   1 atggcctcaa ggagcctctc ctatacacaa ctcctaggcc tcctgtcctt tatactcctc
  61 ttggtcacaa ccaccactat ggcggttcgt gttggagtca ttcttcataa gccttctgct
 121 ccaactcttc ctgttttcag agaagccccg gcttttcgaa acggtgatca atgcgggact
 181 cgtgaggctg atcagattca tatcgccatg actctcgaca caaactacct ccgtggcaca
 241 atggctgccg ttttgtctct ccttcaacat tccacttgcc ctgaaaacct ctcttttcat
 301 ttcctgtccc ttcctcattt cgaaaacgac cttttcacca gcatcaaatc aacctttcct
 361 tacctaaact tcaagattta tcagtttgat ccaaacctcg tccgcagcaa gatatcgaaa
 421 tccatcaggc aagcccttga tcagcctctt aactacgcaa gaatctacct cgcggatatc
 481 atccctagca gcgttgacag gatcatctac ttagactcag acctcgttgt ggtagacgac
 541 atagagaagc tgtggcatgt ggagatggaa ggtaaagtgg tggctgctcc cgagtactgc
 601 cacgcaaact tcacccatta tttcacaaga actttctggt cagacccggt attggtcaaa
 661 gttcttgaag gaaaacgtcc gtgttatttc aacacagggg tgatggttgt ggatgtaaac
 721 aaatggagga aaggaatgta tacacagaag gtagaagagt ggatgacaat tcagaagcag
 781 aagaggatat accatttggg atcattacct ccgtttctgc tgatattcgc cggtgatata
 841 aaagcggtta atcataggtg gaaccagcat ggtctaggag gtgataattt cgaaggaaga
 901 tgtagaacgt tgcatccggg accgataagt cttcttcact ggagtggaaa agggaagcca
 961 tggttaagac tagattcaag gaagccttgt atcgttgatc atctatgggc accgtatgat
1021 ctgtaccgtt catcaagaca ttcattagaa gagtag
```

Amino Acid Sequence of Sequence #21: (SEQ ID NO: 42)
Genebank ID# NP_187277
Positions 1-351.

```
   1 masrslsytq llgllsfill lvttttmavr vgvilhkpsa ptlpvfreap afrngdqcgt
  61 readqihiam tldtnylrgt maavlsllqh stcpenlsfh flslphfend lftsikstfp
 121 ylnfkiyqfd pnlvrskisk sirqaldqpl nyariyladi ipssvdriiy ldsdlvvvdd
 181 iekiwhveme gkvvaapeyc hanfthyftr tfwsdpvlvk vlegkrpcyf ntgvmvvdvn
 241 kwrkgmytqk veewmtiqkq kriyhlgslp pfllifagdi kavnhrwnqh glggdnfegr
 301 crtlhpgpis llhwsgkgkp wlrldsrkpc ivdhlwapyd lyrssrhsle e
```

Sequence #22 (SEQ ID NO:43)

Gene name: At3g28340
GeneBank accession # for reference: NM_113753, GI:30689155
Nucleotide sequence of Sequence #22:
Positions 1-1098 of CDS of NM_113753.

```
   1 atgatgtctg gttcaagatt agcctctaga ctaataataa tcttctcaat aatctccaca
  61 tctttcttca ccgttgaatc gattcgacta ttccctgatt cattcgacga tgcatcttca
 121 gatttaatgg aagctccagc atatcaaaac ggtcttgatt gctctgtttt agccaaaaac
 181 agactcttgt tagcttgtga tccatcagct gttcatatag ctatgactct agatccagct
 241 tacttgcgtg gcacggtatc tgcagtacat tccatcctca aacacacttc ttgccctgaa
 301 aacatcttct tccacttcat tgcttcgggt acaagtcagg gttccctcgc caagacccta
 361 tcctctgttt ttccttcttt gagtttcaaa gtctatacct ttgaagaaac cacggtcaag
 421 aatctaatct cttcttctat aagacaagct cttgatagtc ctttgaatta cgcaagaagc
 481 tacttatccg agattctttc ttcgtgtgtt agtcgagtga tttatctcga ttcggatgtg
 541 attgtggtcg atgatattca gaaactatgg aagatttctt tatccgggtc aagaacaatc
 601 ggtgcaccag agtattgcca cgcaaatttc accaaatact tcacagatag tttctggtcc
 661 gatcaaaaac tctcgagtgt cttcgattcc aagactcctt gttatttcaa cacaggagtg
 721 atggttatcg atttagagcg atggagagaa ggagattaca cgagaaagat cgaaaactgg
 781 atgaagattc agaaagaaga taagagaatc tacgaattgg gttctttacc accgtttctt
 841 ctagtgtttg gtggtgatat tgaagctatt gatcatcaat ggaaccaaca cggtctcggt
 901 ggagacaaca ttgtgagtag ttgtagatct ttgcatcctg gtccggttag tttgatacat
 961 tggagtggta aagggaagcc atgggttagg cttgatgatg gtaagccttg tccaattgat
1021 tatctttggg ctccttatga tcttcacaag tcacagaggc agtatcttca atacaatcaa
1081 gagttagaaa ttctttga
```

Amino Acid Sequence of Sequence #22: (SEQ ID NO: 44)
Genebank ID# NP_189474
Positions 1-365.

```
   1 mmsgsrlasr liiifsiist sfftvesirl fpdsfddass dlmeapayqn gldcsvlakn
  61 rlllacdpsa vhiamtldpa ylrgtvsavh silkhtscpe niffhfiasg tsqgslaktl
 121 ssvfpslsfk vytfeettvk nlisssirqa ldsplnyars ylseilsscv srviyldsdv
 181 ivvddiqklw kislsgsrti gapeychanf tkyftdsfws dqklssvfds ktpcyfntgv
 241 mvidlerwre gdytrkienw mkiqkedkri yelgslppfl lvfggdieai dhqwnqhglg
 301 gdnivsscrs lhpgpvslih wsgkgkpwvr lddgkpcpid ylwapydlhk sqrqylqynq
 361 eleil
```

Sequence #23 (SEQ ID NO:45)

Gene name: At3g50760
GeneBank accession # for reference: NM_114936, GI:18409176
Nucleotide sequence of Sequence #23:
Positions 1-1026 of CDS of NM_114936.

```
   1 atgcactcga agtttatatt atatctcagc atcctcgccg tattcaccgt ctctttcgcc
```

61 ggcggcgaga gattcaaaga agctccaaag ttcttcaact ccccggagtg tctaaccatc
121 gaaaacgatg aagatttcgt ttgttcagac aaagccatcc acgtggcaat gaccttagac
181 acagcttacc tccgtggctc aatggccgtg attctctccg tcctccaaca ctcttcttgt
241 cctcaaaaca ttgttttcca cttcgtcact tcaaaacaaa gccaccgact ccaaaactac
301 gtcgttgctt cttttcccta cttgaaattc cgaatttacc cttacgacgt agccgccatc
361 tccggcctca tctcaacctc catccgctcc gcgctagact ctccgctaaa ctacgcaaga
421 aactacctcg ccgacattct tcccacgtgc ctctcacgtg tcgtatacct agactcagat
481 ctcatactcg tcgatgacat ctccaagctc ttctccactc acatccctac cgacgtcgtt
541 ttagccgcgc ctgagtactg caacgcaaac ttcacgactt actttactcc gacgttttgg
601 tcaaaccctt ctctctccat cacactatcc ctcaaccgcc gtgctacacc gtgttacttc
661 aacaccggag tgatggtcat cgagttaaag aaatggcgag aaggagatta cacgaggaag
721 atcatagagt ggatggagtt acaaaaacgg ataagaatct acgagttagg ctctttacca
781 ccgtttttac ttgtcttcgc cggaaacata gctccggtag atcaccggtg gaaccaacac
841 ggtttaggag gagataattt tagaggactg tgtcgagatt tgcatccagg tccagtgagt
901 ttgttgcatt ggagtgggaa agggaagcca tgggtaaggt tagatgatgg tcgaccttgc
961 ccgcttgatg cactttgggt tccatatgat ttgttagagt cacggttcga ccttatcgag
1021 agttaa

Amino Acid Sequence of Sequence #23: (SEQ ID NO: 46)
Genebank ID# NP_190645
Positions 1-341.

1 mhskfilyls ilavftvsfa ggerfkeapk ffnspeclti endedfvcsd kaihvamtld
61 taylrgsmav ilsvlqhssc pqnivfhfvt skqshrlqny vvasfpylkf riypydvaai
121 sglistsirs aldsplnyar nyladilptc lsrvvyldsd lilvddiskl fsthiptdvv
181 laapeycnan fttyftptfw snpslsitls lnrratpcyf ntgvmvielk kwregdytrk
241 iiewmelqkr iriyelgslp pfllvfagni apvdhrwnqh glggdnfrgl crdlhpgpvs
301 llhwsgkgkp wvrlddgrpc pldalwvpyd llesrfdlie s

Sequence #24 (SEQ ID NO:47)

Gene name: At3g62660
GeneBank accession # for reference: NM_116131, GI:30695642
Nucleotide sequence of Sequence #24:
Positions 1-1086 of CDS of NM_116131.

1 atgctttgga tcatgagatt ctccggttta ttctccgccg ctttggttat catcgtcctc
61 tctccttctc tccaatcgtt tcctccagct gaagctatca gatcctctca tctcgacgct
121 tacctccgtt tcccctcctc cgatccaccg ccgcatagat tctccttcag aaaagctcct
181 gttttccgca atgccgccga ttgcgccgcc gcagatatcg attccggcgt ctgtaaccct
241 tccttggtcc acgtcgcgat tactctcgat ttcgagtacc tgcgtggctc aatcgccgcc
301 gttcattcga ttctcaagca ctcgtcgtgt cccgagagcg tcttcttcca tttcctcgtc
361 tccgagactg acctagaatc cttgattcgt tcgacttttc ccgaattgaa attaaaggtt
421 tactacttcg atccggagat tgtacggacg ctgatctcaa cctccgtgag acaagcgctc
481 gagcagccgt tgaattacgc tagaaattac ctagctgacc ttctcgagcc ttgcgtgcgt
541 cgcgtgatct acctagattc cgatctaatc gtcgtcgacg acatcgcaaa gctctggatg
601 acgaaactgg gatcgaaaac gatcggagct cccgagtact gtcacgcgaa cttcacaaag
661 tatttcacac cggcgttctg gtccgacgag aggttctccg gagctttctc cgggaggaaa

721 ccgtgctact tcaacacggg agtgatggtg atggatctag agagatggag gcgcgtaggg
781 tacacggagg tgatagagaa atggatggag attcagaaga gtgataggat ttacgagctg
841 ggatcattgc cgccgttctt gttggtgttc gccggagaag tagctccgat agagcatcgg
901 tggaaccagc atgggcttgg tggagataac gtgagaggaa gctgtagaga tttacatccc
961 ggtccggtta gcttgcttca ttggtccggt agtggtaaac cgtggtttcg gttagattcg
1021 agacggcctt gtccacttga tactctttgg gcaccttatg atttgtatgg acactactct
1081 cgctga

Amino Acid Sequence of Sequence #24: (SEQ ID NO: 48)
Genebank ID# NP_191825
Positions 1-361.

1 mlwimrfsgl fsaalviivl spslqsfppa eairsshlda ylrfpssdpp phrfsfrkap
61 vfrnaadcaa adidsgvcnp slvhvaitld feylrgsiaa vhsilkhssc pesvffhflv
121 setdleslir stfpelklkv yyfdpeivrt listsvrqal eqplnyarny ladllepcvr
181 rviyldsdli vvddiaklwm tklgsktiga peychanftk yftpafwsde rfsgafsgrk
241 pcyfntgvmv mdlerwrrvg yteviekwme iqksdriyel gslppfllvf agevapiehr
301 wnqhglggdn vrgscrdlhp gpvsllhwsg sgkpwfrlds rrpcpldtlw apydlyghys
361 r

Sequence #25 (SEQ ID NO:49)

Gene name: At4g02130
GeneBank accession # for reference: NM_116445, GI:18411845
Nucleotide sequence of Sequence #25:
Positions 1-1041 of CDS of NM_116445.

1 atgctttgga taacgagatt tgctggatta ttctccgccg cgatggcagt gatcgtgtta
61 tctccgtcgc ttcagtcatt tcctccggcg gcggcaatcc gttcttctcc atcaccgatc
121 ttcagaaaag ctccagcggt gttcaacaac ggcgacgaat gtctctcctc cggcggcgtc
181 tgcaatccgt cgttggtcca cgtggcgatc acgttagacg tagagtacct gcgtggctca
241 atcgcagccg ttaactcgat ccttcagcac tcggtgtgtc cagagagcgt cttcttccac
301 ttcatcgccg tctccgagga aacaaacctg ttggagtcgc tggtgagatc ggttttcccg
361 agactgaaat tcaatattta cgattttgcc cctgagacag ttcgtggttt gatttcttct
421 tccgtgagac aagctctcga gcagcctctg aactacgcta gaagctactt agcggatctg
481 ctggagcctt gtgttaaccg tgtcatatac ttggattcgg atcttgtcgt cgtcgatgac
541 atcgctaagc tttggaaaac tagcctaggc tcgaggataa tcggagctcc ggagtattgt
601 cacgcgaatt tcacgaaata cttcaccgga ggattctggt cggaggagag attctccggt
661 acctttagag ggaggaagcc atgttacttc aacacaggtg tgatggtgat agatcttaag
721 aaatggagaa gaggtggtta cacgaaacgt atcgagaaat ggatggagat tcagagaaga
781 gagaggattt acgaactagg ctcgcttcca ccgtttcttc tagttttctc cggtcacgtg
841 gctcccatct ctcaccggtg gaaccagcat ggacttggtg gtgacaatgt tagaggtagc
901 tgtcgtgatt tgcatcctgg tcctgtgagt ttgctgcatt ggtctggtag tggcaagccc
961 tggataagac tcgattccaa acggccttgt cccttagacg cattatggac gccttacgac
1021 ttgtatcgac attcgcattg a

Amino Acid Sequence of Sequence #25: (SEQ ID NO: 50)
Genebank ID# NP_192122
Positions 1-346.

```
  1 mlwitrfagl fsaamavivl spslqsfppa aairsspspi frkapavfnn gdeclssggv
 61 cnpslvhvai tldveylrgs iaavnsilqh svcpesvffh fiavseetnl leslvrsvfp
121 rlkfniydfa petvrgliss svrqaleqpl nyarsyladl lepcvnrviy ldsdlvvvdd
181 iaklwktslg sriigapeyc hanftkyftg gfwseerfsg tfrgrkpcyf ntgvmvidlk
241 kwrrggytkr iekwmeiqrr eriyelgslp pfllvfsghv apishrwnqh glggdnvrgs
301 crdlhpgpvs llhwsgsgkp wirldskrpc pldalwtpyd lyrhsh
```

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The amino acids which occur in the various amino acid sequences referred to in the specification have their usual three- and one-letter abbreviations routinely used in the art: A, Ala, Alanine; C, Cys, Cysteine; D, Asp, Aspartic Acid; E, Glu, Glutamic Acid; F, Phe, Phenylalanine; G, Gly, Glycine; H, His, Histidine; I, Ile, Isoleucine; K, Lys, Lysine; L, Leu, Leucine; M, Met, Methionine; N, Asn, Asparagine; P, Pro, Proline; Q, Gln, Glutamine; R, Arg, Arginine; S, Ser, Serine; T, Thr, Threonine; V, Val, Valine; W, Try, Tryptophan; Y, Tyr, Tyrosine.

A protein is considered an isolated protein if it is a protein isolated from the plant, or from a host cell in which it is recombinantly produced. It can be purified or it can simply be free of other proteins and biological materials with which it is associated in nature.

An isolated nucleic acid is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA which has the sequence

of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding or noncoding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (i) DNA molecules, (ii) transformed or transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

As used herein expression directed by a particular sequence is the transcription of an associated downstream sequence. If appropriate and desired for the associated sequence, there the term expression also encompasses translation (protein synthesis) of the transcribed RNA. When expression of a sequence of interest is "up-regulated," the expression is increased. With reference to up-regulation of expression of a sequence of interest operably linked to a transcription regulatory sequence, expression is increased.

In the present context, a promoter is a DNA region which includes sequences sufficient to cause transcription of an associated (downstream) sequence. The promoter may be regulated, i.e., not constitutively acting to cause transcription of the associated sequence. If inducible, there are sequences present which mediate regulation of expression so that the associated sequence is transcribed only when an inducer molecule is present in the medium in or on which the organism is cultivated. In the present context, a transcription regulatory sequence includes a promoter sequence and can further include cis-active sequences for regulated expression of an associated sequence in response to environmental signals.

One DNA portion or sequence is downstream of second DNA portion or sequence when it is located 3' of the second sequence. One DNA portion or

sequence is upstream of a second DNA portion or sequence when it is located 5' of that sequence.

One DNA molecule or sequence and another are heterologous to another if the two are not derived from the same ultimate natural source. The sequences may be natural sequences, or at least one sequence can be designed by man, as in the case of a multiple cloning site region. The two sequences can be derived from two different species or one sequence can be produced by chemical synthesis provided that the nucleotide sequence of the synthesized portion was not derived from the same organism as the other sequence.

An isolated or substantially pure nucleic acid molecule or polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native transcription regulatory sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter effects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be operably linked even at a distance, i.e., even if not contiguous.

The term recombinant polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other sequences, for example, those from other species or other strains. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide probes may be labeled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a protein of interest are incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the construct is suitable for replication in a unicellular host, such as *A. pullulans* or a bacterium, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cell. Commonly used prokaryotic hosts include strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or a pseudomonad, may also be used. Eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian, mammalian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors influence the choice of the host cell.

The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.*, **22**: 1859-1862 or the triester method according to Matteuci *et al.* (1981) *J.*

Am. Chem. Soc., 103:3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook *et al.* (1989) vide infra; Ausubel *et al.* (Eds.) (1995) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York; and Metzger *et al.* (1988) *Nature*, **334**: 31-36. Many useful vectors for expression in bacteria, yeast, fungal, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors

may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

Recombinant host cells, in the present context, are those which have been genetically modified to contain an isolated DNA molecule of the instant invention. The DNA can be introduced by any means known to the art which is appropriate for the particular type of cell, including without limitation, transformation, lipofection or electroporation.

It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences which code for the polypeptide or protein of interest are included in this invention.

Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the DNA sequences which will not significantly change activity of the amino acid sequences of the peptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of the regulated promoter region. The skilled artisan will understand that the sequence of the exemplified sequence can be used to identify and isolate additional, nonexemplified nucleotide sequences which are functionally equivalent to the sequences given.

Mutational, insertional, and deletional variants of the disclosed nucleotide sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the exemplified primer sequences so long as the variants have substantial sequence homology with the original sequence. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant polynucleotide to function in the same capacity as the polynucleotide from which the probe was derived. Preferably, this homology is greater than 80%, more preferably, this homology is greater than 85%, even more preferably this homology is greater than 90%, and most preferably, this homology is greater than 95%. The degree of homology or identity needed for the variant to function in its intended capacity depends upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are equivalent in function or are designed to improve the function of the sequence or otherwise provide a methodological advantage.

Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art [see Mullis, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159; Saiki *et al.* (1985) *Science* 230:1350-1354]. PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA template produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a few hours. By using a thermostable DNA polymerase such as the *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes which can be used are known to those skilled in the art.

It is well known in the art that the polynucleotide sequences of the present invention can be truncated and/or mutated such that certain of the resulting fragments and/or mutants of the original full-length sequence can retain the desired characteristics of the full-length sequence. A wide variety of restriction enzymes which are suitable for generating fragments from larger nucleic acid molecules are well known. In addition, it is well known that *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA. See, for example, Maniatis (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, pages 135-139, incorporated herein by reference. See also Wei *et al.* (1983 *J. Biol. Chem.* **258**:13006-13512. By use of *Bal*31 exonuclease (commonly referred to as "erase-a-base" procedures), the ordinarily skilled artisan can remove nucleotides from either or both ends of the subject nucleic acids to generate a wide spectrum of fragments which are functionally equivalent to the subject nucleotide sequences. One of ordinary skill in the art can, in this manner, generate hundreds of fragments of controlled, varying lengths from locations all along the original molecule. The ordinarily skilled artisan can routinely test or screen the generated fragments for their characteristics and determine the utility of the fragments as taught herein. It is also well known that the mutant sequences of the full length sequence, or fragments thereof, can be easily produced with site directed mutagenesis. See, for example, Larionov, O.A. and Nikiforov, V.G. (1982) *Genetika* **18**(3):349-59; Shortle, D, DiMaio, D., and Nathans, D. (1981) *Annu. Rev. Genet.* **15**:265-94; both incorporated herein by reference. The skilled artisan can routinely produce deletion-, insertion-, or substitution-type mutations and identify those resulting mutants which contain the desired characteristics of the full length wild-type sequence, or fragments thereof, i.e., those which retain promoter activity and also provide transcription of downstream sequence.

Following the teachings herein and using knowledge and techniques well known in the art, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein without the expense of undue experimentation.

As used herein percent sequence identity of two nucleic acids is determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA*

87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* **90**:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* **215**:402-410. BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST is used as described in Altschul *et al.* (1997) *Nucl. Acids. Res.* **25**:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) are used. See, for example, the National Center for Biotechnology Information website on the internet.

Techniques and agents for introducing and selecting for the presence of heterologous DNA in plant cells and/or tissue are well-known. Genetic markers allowing for the selection of heterologous DNA in plant cells are well-known, e.g., genes carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin. The marker allows for selection of successfully transformed plant cells growing in the medium containing the appropriate antibiotic because they will carry the corresponding resistance gene. In most cases the heterologous DNA which is inserted into plant cells contains a gene which encodes a selectable marker such as an antibiotic resistance marker, but this is not mandatory. An exemplary drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers *et al.*, Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988).

Techniques for genetically engineering plant cells and/or tissue with an expression cassette comprising an inducible promoter or chimeric promoter fused to a heterologous coding sequence, including possibly an antisense DNA construct and/or a DNA construct designed to elicit double-stranded RNA-mediated gene silencing, followed by a transcription termination sequence are to be introduced into the plant cell or tissue by *Agrobacterium*- mediated transformation, electroporation, microinjection, particle bombardment or other techniques known to the art. The

expression cassette advantageously further contains a marker allowing selection of the heterologous DNA in the plant cell, e.g., a gene carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamicin, or bleomycin.

A DNA construct carrying a plant-expressible gene or other DNA of interest can be inserted into the genome of a plant by any suitable method. Such methods may involve, for example, the use of liposomes, electroporation, diffusion, particle bombardment, microinjection, gene gun, chemicals that increase free DNA uptake, e.g., calcium phosphate coprecipitation, viral vectors, and other techniques practiced in the art. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, such as those disclosed by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 120,516 (Schilperoort *et al.*). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells.

The choice of vector in which the DNA of interest is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication, protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. The vector desirably includes a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition, preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells.

Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline, among other selective agents. The neomycin phosphotransferase gene has the advantage that it is expressed in eukaryotic as well as prokaryotic cells.

Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, CA) and pPL, pK and K223 available from Pharmacia (Piscataway, NJ), and pBLUESCRIPT and pBS available from Stratagene (La Jolla, CA). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis *et al.*, eds., Cold Spring Harbor Press (1989) and the Lambda ZAP vectors available from Stratagene (La Jolla, CA). Other exemplary vectors include pCMU [Nilsson *et al.* (1989) *Cell* **58**:707]. Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/Kb and pCMUII used in various applications herein are modifications of pCMUIV [Nilsson, (1989) supra].

Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells and capable of directing stable integration within the host plant cell include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.* (1987) *Meth. in Enzymol.* **153**:253-277, and several other expression vector systems known to function in plants. See for example, Verma *et al.*, No. WO87/00551; Cocking and Davey (1987) *Science* **236**:1259-1262.

A transgenic plant can be produced by any means known to the art, including but not limited to *Agrobacterium tumefaciens*-mediated DNA transfer, preferably with a disarmed T-DNA vector, electroporation, direct DNA transfer, and particle bombardment [See Davey *et al.* (1989) *Plant Mol. Biol.* **13**:275; Walden and Schell (1990) *Eur. J. Biochem.* **192**:563; Joersbo and Burnstedt (1991) *Physiol. Plant.* **81**:256; Potrykus (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**:205; Gasser and Fraley (1989) *Science* 244:1293; Leemans (1993) *Bio/Technology* **11**:522; Beck *et al.* (1993) *Bio/Technology* **11**:1524; Koziel *et al.* (1993) *Bio/Technology* **11**:194; Vasil *et al.* (1993) *Bio/Technology* **11**:1533 and Gelvin, S.B. (1999) *Curr. Opin. Biotech.* **9**:227-232]. Techniques are well-known to the art for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues.

Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook *et al.* (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis *et al.* (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* **218**, Part I; Wu (ed.) (1979) *Meth. Enzymol.* **68**; Wu *et al.* (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* **65**; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, **Vols. 1-4**, Plenum Press, New York; and Ausubel *et al.* (1992) *Current Protocols in Molecular Biology*, Greene/Wiley, New York, NY. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All references cited in the present application are incorporated in their entirety herein by reference to the extent not inconsistent herewith.

References

1. Liljebjelke, K. et al., Enzymatic synthesis and purification of uridine diphosphate [^{14}C]galacturonic acid: a substrate for pectin biosynthesis, *Anal. Biochem.* **225**:296-304 (1995).

2. Doong, R.L. *et al.*, Cell free synthesis of pectin: identification and partial characterization of polygalacturonate 4-alpha-galacturonosyltransferase and its products from membrane preparations of tobacco (*Nicotiana tabacum* L. cv samsun) cell suspension cultures, *Plant Physiol.* **109**:141-152 (1995).

3. Doong, R.L. *et al.* Solubilization and characterization of a galacturonosyltransferase that synthesizes the pectic polysaccharide homogalacturonan, *The Plant Journal* **13**:363-374 (1998).

4. Scheller, H.V. *et al.*, Pectin biosynthesis: a solubilized galacturonosyltransferase from tobacco catalyzes the transfer of galacturonic acid from UDP-galacturonic acid onto the non-reducing end of homogalacturonan, Planta **207**:512-517 (1999).

5. Mohnen, D. *et al.*, A multi-enzyme approach to study pectin biosynthesis, *Annual Meeting of the American Society of Plant Physiology, July 24-July 28* Abst. No. **203**:65(1999).(Abstract)

6. Mohnen, D., Biosynthesis of pectins and galactomannans, in: "Comprehensive Natural Products Chemistry, Vol. 3, Carbohydrates and Their Derivatives including Tannins, Cellulose, and Related Lignins", B.M. Pinto., ed., *Elsevier*, Oxford, pp. 497-527 (1999).

7. Sterling, J. *et al.*, The catalytic site of the pectin biosynthetic enzyme alpha-1,4-galacturonosyltransferase (GalAT) is located in the lumen of the Golgi, *Plant Physiol.* **127**:360-371 (2001).

8. Ridley, B.L. *et al.*, Pectins: structure, biosynthesis, and oligogalacturonide-related signaling, *Phytochemistry* **57**:929-967 (2001).

9. Mohnen, D., Biosynthesis of pectins, in: "Pectins and their Manipulation", G.B. Seymour *et al.*, Blackwell Publishing and CRC Press, Oxford, pp. 52-98 (2002).

10. Villemez, C.L. *et al.*, Properties of a polygalacturonic acid-synthesizing enzyme system from *Phaseolus aureus* seedlings. *Arch. Biochem. Biophys.* **116**:446-452 (1966).

11. Kauss, H. *et al.*, Cooperation of enzymes responsible for polymerization and methylation in pectin biosynthesis. *Z. Naturforsch.* **24**:28-33 (1969).

12. Lin, T.-Y. *et al.*, Substrate specificity in pectin synthesis. *Biochem. Biophys. Res. Commun.* **22**:650-657 (1966).

13. Bolwell, G. P, *et al.*, Decrease of polygalacturonic acid synthase during xylem differentiation in sycamore. *Phytochemistry* **24**:699-702 (1985).

14. Takeuchi, Y. et al., In vitro biosynthesis of homogalacturonan by a membrane-bound galacturonosyltransferase from epicotyls of azuki bean, *Biosci. Biotech. Biochem.* **65**:1519-1527 (2001).

15. Akita, K. *et al.*, Successive glycosyltransfer activity and enzymatic characterization of pectic polygalacturonate 4-α-galacturonosyltransferase solubilized from pollen tubes of *Petunia axillaris* using pyridylaminated oligogalacturonates as substrates, *Plant Physiol.* **130**:374-379 (2002).

16. Reithmeier, R.A.F. *et al.*, Intrinsic membrane protein structure: principles and prediction, in: "The Structure of Biological Membranes", P. Yeagle., ed., CRC Press, Boca Raton, pp. 337-393 (1992).

17. Northcote, D.H., The Golgi apparatus. *Endeavor* **30**:26-33 (1971).

18. Northcote, D.H. *et al.,* A function of the Golgi Apparatus in polysaccharide synthesis and transport in the root-cap cells of wheat, *Biochem. J.* **98**:159-167 (1966).

19. Harrism, P.J. *et al.*, Polysaccharide formation in plant golgi bodies. Biochim. Biophys. Acta **237**:56-64 (1971).

20. Stoddart, R.W. *et al.*, Metabolic relationships of the isolated fractions of the pectic substances of actively growing sycamore cells. *Biochem. J.* **105**:45-59 (1967).

21. Moore, P.J. *et al.*, Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the golgi apparatus of plants. *J. Cell Biol.* **112**:589-602 (1991).

22. Staehelin, L.A. *et al.*, The plant Golgi apparatus: structure, functional organization and trafficking mechanisms, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**:261-288 (1995).

23. Willats, W.G.T. *et al.*, Making and using antibody probes to study plant cell walls, *Plant Physiol. Biochem.* **38**:27-36 (2000).

24. Goubet, F. *et al.,* Subcellular localization and topology of homogalacturonan methyltransferase in suspension-cultured *Nicotiano tabacum* cells, *Planta* **209**:112-117 (1999).

25. Vannier, M.P. *et al.,* Localization of methyltransferase activities throughout the endomembrane complex system of flax (*Linum usitatissimum* L) hypocotyls, *Biochem. J.* **286**:863-868 (1992).

26. Bourlard, T. *et al.,* Various pectin methyltransferase activities with affinity for low and highly methylated pectins, *Plant Cell Physiol.* **38**:259-267 (1997).

27. O'Neill, M. *et al.*, The pectic polysaccharides of primary cell walls, in: "Methods in Plant Biochemistry, Volume 2", P.M. Dey., ed., Academic Press, London, pp. 415-441 (1990).

28. Lau, J.M., *et al.*, Structure of the backbone of rhamnogalacturonan I, a pectic polysaccharide in the primary cell walls of plants, Carbohydr. Res. **137**:111-125 (1985).

29. Eda, S. *et al.*, A pectic polysaccharide from cell walls of tobacco (*Nicotiana tabacum*) mesophyll, Carbohydr. Res. **158**:205-216 (1986).

30. Carpita, N.C. *et al.*, Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth, *Plant J.* **3**:1-30 (1993).

31. O'Neill, M.A. *et al.*, Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester - in vitro conditions for the formation and hydrolysis of the dimer, *J. Biol. Chem.* **272**:3869(1997).

32. Schols, H.A. *et al.*, A xylogalacturonan subunit present in the modified hairy regions of apple pectin, Carbohydr. Res. **279**:265-279 (1995).

33. Kikuchi, A. *et al.*, A xylogalacturonan whose level is dependent on the size of cell clusters is present in the pectin from cultured carrot cells, *Planta* **200**:369-372 (1996).

34. Yu, L. *et al.*, Partial characterization of xylogalacturonans from cell walls of ripe watermelon fruit: Inhibition of endopolygalacturonase activity by xylosylation, in: "Pectins and Pectinases", J. Visser *et al.*, *Elsevier*, Amsterdam, pp. 79-88 (1996).

35. Aspinall, G.O., Chemistry of cell wall polysaccharides. in: "The Biochemistry of Plants, Vol.3.", J. Preiss., ed., Academic Press, New York, pp. 473-500 (1980).

36. Watson, R.R. *et al.*, Chemistry and biochemistry of apiose, *Adv. Carbohydr. Chem. Biochem.* **31**:135-184 (1975).

37. Hart, D.A. *et al.*, Isolation and partial characterization of apiogalacturonans from the cell wall of *Lemna minor*, *Biochem. J.* **116**:569-579 (1970).

38. Schols, H.A. *et al.*, Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process, *Carbohydr. Res.* **206**:117-129 (1990).

39. Mohnen, D. *et al.*, Cell free synthesis of the pectic polysaccharide homogalacturonan, in: "Pectins and Pectinases", J. Visser *et al.*, *Elsevier Science* B.V. Amsterdam, pp. 109-126 (1996).

40. Willats, W.G.T. *et al.*, Pectin: cell biology and propects for functional analysis, *Plant Mol. Biol.* **47**:9-27 (2001).

41. An, J. *et al.*, Isolation and structural characterization of alpha-D-glucosyluronic acid and 4-*O*-methyl alpha-D-glucosyluronic acid-containing oligosaccharides from the cell-wall pectic polysaccharide, rhamnogalacturonan I, *Carbohydr. Res.* **252**:235-243 (1994).

42. An, J. *et al.*, Isolaton and structural characterization of endo-rhamnogalacturonase-generated fragments of the backbone of rhamnogalacturonan I, *Carbohydr. Res.* **264**:83-96 (1994).

43. O'Neill, M.A. *et al.*, Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth, *Science* **294**:846-849 (2001).

44. Atkinson, R.G. *et al.*, Overexpression of polygalacturonase in transgenic apple trees leads to a range of novel phenotypes involving changes in cell adhesion, *Plant Physiol.* **129**:122-133 (2002).

45. Mohnen, D. *et al.*, Cell wall carbohydrates as signals in plants, *Sem. Cell Biol.* **4**:93-102 (1993).

46. Côté, F. *et al.*, Oligosaccharide elicitors in host-pathogen interactions generation, perception, and signal transduction, in: "Plant-Microbe Interactions", B.B. Biswas *et al.*, Plenum Press, New York, pp. 385-432 (1998).

47. Mollet, J.-C. *et al.*, A lily stylar pectin is necessary for pollen tube adhesion to an in vitro stylar matrix, *Plant Cell* **12**:1737-1749 (2000).

48. Western, T.L. *et al.*, Isolation and characterization of mutants defective in seed coat mucilage secretory cell development in Arabidopsis, *Plant Physiol.* **127**:998-1011 (2001).

49. Willats, W.G.T. *et al.*, In-situ analysis of pectic polysaccharides in seed mucilage and at the root surface of *Arabidopsis thaliana*, *Planta* **213**:37-44 (2001).

50. González-Carranza, Z.H. *et al.*, Temporal and spatial expression of a polygalacturonase during leaf and flower abscission in oilseed rape and Arabidopsis, *Plant Physiol.* **128**:534-543 (2002).

51. Brown, K., Xylem may direct water where it's needed, *Science* **291**:571-572 (2001).

52. Fry, S. *et al.*, Oligosaccharides as Signals and Substrates in the Plant Cell Wall, *Plant Physiol.* **103**:1-5 (1993).

53. Shibuya, N. *et al.*, Oligosaccharide signalling for defence responses in plant, *Physiol. Mol. Plant Pathol.* **59**:223-233 (2001).

54. Skjot, M. *et al.*, Direct interference with rhamnogalacturonan I biosynthesis in Golgi vesicles, *Plant Physiol.* **129**:95-102 (2002).

55. Bouton, S. *et al.*, QUASIMODO1 encodes a putative membrane-bound glycosyltransferase required for normal pectin synthesis and cell adhesion in Arabidopsis, *Plant Cell* **14**:2577-2590 (2002).

56. Edwards, M.E. *et al.*, Molecular characterisation of a membrane-bound galactosyltransferase of plant cell wall matrix polysaccharide biosynthesis, *Plant J.* **19**:691-697 (1999).

57. Perrin, R.M. *et al.*, Xyloglucan fucosyltransferase, an enzyme involved in plant cell wall biosynthesis, *Science* **284**:1976-1979 (1999).

58. Faik, A. *et al.*, An *Arabidopsis* gene encoding an alpha-xylosyltransferase involved in xyloglucan biosynthesis, *Proc. Natl. Acad. Sci. USA* **99**:7797-7802 (2002).

59. Delmer, D.P., A hot mutant for cellulose synthesis, *Trends in Plant Science* **3**:164 (1998).

60. Orellana, A. *et al.*, Enzymatic synthesis and purification of [^{3}H] uridine diphosphate galacturonic acid for use in studying Golgi-localized transporters, *Analytical Biochemistry* **272**:224-231 (1999).

61. Crombie, H.J. *et al.*, A homogalacturonan synthase from mung bean hypocotyls, Cell Wall '01 - 9th International Cell Wall Meeting, September 2-7, 2001 Toulouse, France 131(2001).(Abstract)

62. Cumming, C.M. *et al.*, A galacturonyltransferase involved in pectin biosynthesis. in: "Cell Walls '86. Proceedings of the Fourth Cell Wall Meeting. Paris - September 10-12, 1986", B. Vian *et al.*, Université Pierre et Marie Curie - Ecole Normale Supérieure. Paris, pp. 360-363 (1986).

63. Sterling, J.D. *et al.*, Development of a filter assay for measuring homogalacturonan:alpha1,4-galacturonosyltransferase activity, (in preparation)

64. Williams, N., Rain forest fragments fare poorly, *Science* **278**:1016(1997).

65. Pagès, S. *et al.*, Changing a single amino acid residue switches processive and non-processive behavior of *Aspergillus niger* endopolygalacturonase I and II, *J. Biol. Chem.* **276**:33652-33656 (2001).

66. Campbell, J.A. *et al.*, A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities, *Biochem. J.* **326**:929-942 (1997).

67. Henrissat, B. *et al.*, Glycoside hydrolases and glycosyltransferases. Families, modules, and implications for genomics. *Plant Physiol* **124**:1515-1519 (2000).

68. Jones, D.T., GenTHREADER: An Efficient and Reliable Protein Fold Recognition Method for Genomic Sequences, *J. Mol. Biol.* **287**:797-815 (1999).

69. McGuffin, L.J. *et al.*, The PSIPRED protein structure prediction server, *Bioinform. Applic. Note.* **16**:404-405 (2000).

70. Persson, K. *et al.*, Crystal structure of the retaining galactosyltransferase LgtC from *Neisseria meningitidis* in complex with donor and acceptor sugar analogs, *Nature Structural Biology* **8**:166-175 (2001).

71. Price, N.J. *et al.*, Plant glycosyltransferases, *Curr. Opin. Plant Biol.* 2001:219-224 (2001).

72. Charnock, S.J. *et al.*, Three-dimensional structures of UDP-sugar glycosyltransferases illuminate the biosynthesis of plant polysaccharides, *Plant Physiol.* **125**:527-531 (2001).

73. Ünligil, U.M. *et al.*, Glycosyltransferase structure and mechanism, *Current Opinion in Structural Biology* **10**:510-517 (2000).

74. Boix, E. *et al.*, Structural basis of ordered binding of donor and acceptor substrates to the retaining glycosyltransferase, alpha-1,3-galactosyltransferase, *J. Biol. Chem.* **277**:28310-28318 (2002).

75. Cabral, C.M. *et al.*, Organizational diversity among distinct glycoprotein endoplasmic reticulum-associated degradation programs, *Mol. Biol. Cell* **13**:2639-2650 (2002).

76. Mallisard, M. *et al.*, Expression of functional soluble forms of human alpha-1,4-galactosyltransferase I, alpha-2,6-sialyltransferase, and alpha-2,6-sialyltransferase, and alpha-1,3-fucosyltransferase VI in the methylotrophic yeast *Pichia pastoris*, Biochem. Biophys. Res. Commun. **267**:169-173 (2000).

77. Romero, P.A. *et al.*, KTR1P is an alpha-1,2-mannosyltransferase of *Saccharomyces cerevisiae* comparison of the enzymatic properties of soluble recombinant KTR1P and KRE2P/MNT1P produced in *Pichia pastoris*, *Biochemical Journal* **321**:289-295 (1997).

78. Hochstrasser, U. *et al.*, Expression of a functional barley sucrose-fructan 6-fructosyltransferase in the methylotrophic yeast *Pichia pastoris*, *Febs Letters* **440**:356-360 (1998).

79. Gallet, P.F. *et al.*, Heterologous expression of an engineered truncated form of human Lewis fucosyltransferase (Fuc-TIII) by the methylotrophic yeast *Pichia pastoris*, *Glycobiology* **8**:919-925 (1998).

80. Moreman, K.W. *et al.*, Topology of mannosidasw II in rat liver membrane and release of the cataytic domain by selective proteolysis, *Jr Biol. Chem* **23**:10945-10951 (1986).

81. Merkle, R.K. *et al.*, Cloning, expression, purification, and charactarization of the murine lysosomal acid alpha-mannosidase, *Biochim. Biophys. Acta* **1336**:132-146 (1997).

82. Liao, Y.F. *et al.*, Cloning, expression, purification, and characterization of the human broad-specificity lysosomal acid alpha-mannosidase, *J. Biol. Chem.* **271**:28348-28358 (1996).

83. Bar-Peled, M. *et al.*, UDP-rhamnose:flavanone-7-*O*-glucoside-2"-*O*-rhamnosyltransferase. Purification and characterization of an enzyme catalyzing the production of bitter compounds in citrus, *J. Biol. Chem.* **266**:20953-20959 (1991).

84. Basu, S.S. *et al.*, A facile enzymatic synthesis of uridine diphospho-[14C]galacturonic acid, *Anal. Biochem.* **280**:173-177 (2000).

85. Quigley, H.F. *et al.*, A non-radioactive gel electrophoresis assay for homogalacturonan alpha-1,4-galacturonosyltransferase, (in preparation).

86. Bao, M. *et al.*, Bovine UDP-*N*-acetylglucosamine:lysosomal-enzyme *N*-acetylglucosamine-1-phosphotransferase. I. Purification and subunit structure, *J. Biol. Chem.* **271**:31437-31445 (1996).

87. Briand, J.P. *et al.*, Application and limitations of the multiple antigen peptide (MAP) system in the production and evaluation of anti-peptide and anti-protein antibodies, *J. Immunol. Methods* **156**:255-265 (1992).

88. Ziehl, V. *et al.*, Polyclonal antibodies directed against synthetic N-terminus of fungal endopolygalacturonase recognizes the native protein and cross-reacts with endopolygalacturonase of a different funal species, Abstract, Pectins and Pectinases meeting, Wageningen, Netherlands, (1995)

89. Harlow, E. *et al.*, Antibodies: A laboratory manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, (1988).

90. Mohnen, D. *et al.*, Hormonal regulation of alpha1,3-glucanase messenger RNA levels in cultured tobacco tissues. *EMBO J.* **4**:1631-1635 (1985).

91. Zhang G.F. *et al.*, Functional compartmentation of the golgi apparatus of plant cells; Immunocytochemical analysis of high-pressure frozen- and freeze-substituted sycamore maple suspension culture cells, *Plant Physiol.* **99**:1070-1083 (1992).

92. Colley, K.J., Golgi localization of glycosyltransferases: more questions than answers, *Glycobiology* **7**:1-13 (1997).

93. Opat, A.S. *et al.*, Trafficking and localization of resident Golgi glycosylation enzymes, *Biochemie* **83**:763-773 (2001).

94. Dirnberger, D. *et al.*, The Golgi localization of Arabidopsis thaliana B1,2-xylosyltransferase in plant cells is dependent on its cytoplasmic and transmembrane sequences, *Plant Mol. Biol.* **50**:273-281 (2002).

95. Munro, S., An investigation of the role of transmembrane domains in golgi protein retention, *The EMBO Journal* **14**:4695-4704 (1995).

96. Nilsson, T. *et al.*, Kin recognition. A model for the retention of Golgi enzymes. *FEBS Lett.* **330**:1-4 (1993).

97. Freshour, G. *et al.*, Developmental and tissue-specific structural alterations of the cell-wall polysaccharides of *Arabidopsis thaliana* Roots, *Plant Physiol.* **110**:1413-1429 (1996).

98. Samuels, A.L. *et al.*, Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants, *J. Cell Biol.* **130**:(1995).

99. Lam, B.C.-H. *et al.*, Role of SH3 Domain-Containing Proteins in Clathrin-Mediated Vesicle Trafficking in Arabidopsis, *Plant Cell* **13**:2499-2512 (2001).

100. Chuang, C.-F. *et al.*, Specific and heritable genetic interference by double-stranded RNA in arabidopsis thaliana, *Proc. Natl. Acad. Sci. USA* **97**:4985-4990 (2000).

101. Bent, A.F. *et al.*, *RPS2* of *Arabidopsis thaliana*: A Leucine-Rich Repeat Class of Plant Disease Resistance Genes, *Science* **265**:1856-1860 (1994).

102. Bieberich, E. *et al.*, Regulation of Ganglioside Biosynthesis by Enzyme Complex Formation of Glycosyltransferases, *Biochem.* **41**:11479-11487 (2002).

103. Ridley, B.L. *et al.*, A method for biotin labeling of biologically active oligogalacturonides using a chemically stable hydrazide linkage, *Anal. Biochem.* **249**:10-19 (1997).

104. Guillaumie, F. *et al.*, Solid-phase biosynthesis and MALDI-TOF mass spectrometry analysis of pectic oligogalacturonides: a new tool to monitor the extension of a homogalacturonan chain, *Carbohydr. Res.* (2002).

105. Yamada, H. *et al.*, Structural Characterization and Antitumor Activity of a Pectic Polysaccharide from the Roots of Angelica acutiloba. *planta medica* **56**:182-186, (1990).

106. Olano-Martin, E. *et al.*, Pectin and pectic-oligosaccharides induce apoptosis in *in vitro* human colonic adenocarcinoma cells. *Anticancer research* **23**:341-346, (2003.).

107. Avivi-Green, C. *et al.*, Pectin-enriched diet affects distribution and expression of apoptosis-cascade proteins in colonic crypts of dimethylhydrazine-treated rats. *Int J Mol Med* **6**:689-698 (2000a).

108. Avivi-Green, C. *et al.*, Apoptosis cascade proteins are regulated in vivo by high intracolonic butyrate concentration: correlation with colon cancer inhibition. *Oncol Res* **12**:83-95 (2000b).

109. Ohno, K. *et al.*, Inhibitory effect of apple pectin and culture condensate of Bifidobacterium longum on colorectal tumors induced by 1,2-dimethylhydrazine in trangenic mice harboring human prototype c-Ha-ras genes. *Exp Anim* **49**:305-307 (2000).

110. Davidson, L.A., *et al.*, Morphodensitometric anaylsis of protein kinase C Bu expression in rat colon: modulation by diet and relation to in situ cell proliferation and apoptosis. *Carcinogenesis* **21**:1513-1519 (2000).

111. Fernandez, M.L., Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose-response study in guinea pigs. *Am J Clin Nutr* **59**: 869-878 (1994).

112. Behall, K. *et al.,* In: ML Fishman and JJ Jen, eds, Chemistry and Function of Pectins, American Chemical Society, Washington, D.C. pp 248-265 (1986).

113. Levitt, N.S. *et al.,* The effect of dietary fiber on glucose and hormone responses to a mixed meal in normal subjects and in diabetic subjects with and without autonomic neuropathy. *Diabetes Care* ***3***: 505-519 (1980).

114. Hayashi, A. *et al.,* Effects of daily oral administration of quercetin chalcone and modified citrus pectin on implanted colon-25 tumor growth in bulb-c mice. *Altern Med Rev* ***5***: S-546-552 (2000).

115. Inohara, H. *et al.*, Effects of natural complex carbohydrates (citrus pectin) on murine melanoma cell properties related to galectin-3 functions. *Glycoconjugates Journal* **11**: 527-532 (1994).

116. Dongowski, G, *et al.*, In: J Visser and AGJ Voragen, eds, Pectins and Pectinases, *Elsevier Science* B.V. Amsterdam, pp 659-666 (1996).

117. Honjo, Y. *et al.,* Expression of Cytoplasmic Galectin-3 as a Prognostic Marker in Tongue Carcinoma. *Clinical Cancer Research.* **6**(12):4635-40, (2000).

118. Honjo, Y. *et al.*, Down-Regulation of Galectin-3 Suppresses Tumorigenicity of Human Breast Carcinoma Cells. *Clinical Cancer Research.* **7**(3):661-8, (2001).

119. Yoshii, T. *et al.*, Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. *Int. J. Oncol.* **18**(4):787-92, (2001).

120. Naik, H. *et al.,* Inhibition of *in vitro* tumor cellendothelial adhesion by modi citrus pectin: a pH modified natural complex carbohydrate (Meeting abstract) *Proc Annul Meet Am Assoc Cancer Res.*;**36**: 377 (1995).

121. Platt, D. *et al.,* Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin. *J Natl Cancer Inst.*; **84**(6):438-442 (1992).

122. Strum, S. *et al.*, International Conference on Diet and Prevention of Cancer (Finland). May (1999).

123. Liu, C. *et al.,* Citrus pectin: characterization and inhibitory effect on fibroblast growth factor-receptor interaction. *J Agric Food Chem* **49**: 3051-3057 (2001).

124. Rolin, C. Pectin. In: RL Whistler and JN BeMiller, eds, Industrial Gums - Polysaccharides and Their Derivatives, Third Ed. Academic Press, San Diego, pp 257-293 (1993).

125. Nangia-Makker, P. *et al.* Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *J. Natl. Cancer Inst.* **94**: 1854-1862 (2002).

126. Dongowski, G *et al.*, Degradation of pectins with different degrees of esterification by *Bacteroides thetaiotaomicron* isolated from human gut flora. Appl *Environ Microbiol* **66**: 1321-1327 (2000).

127. Pienta, K.J. *et al.*, Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin, *J. Natl. Cancer Inst.* **87**:348-353 (1995).

128. Zwieniecki, M.A. *et al.*, Hydrogel control of xylem hydraulic resistance in plants, *Science* **291**:1059-1062 (2001).

129. Skjot, M. *et al.*, Direct interference with rhamnogalacturonan I biosynthesis in Golgi vesicles, *Plant Physiol.* **129**:95-102 (2002).

130. Iwai, H., A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem, *Proc. Natl. Acad. Sci. USA* **99**:16319-16324 (2002).

131. York, W.S. *et al.*, Isolation and characterization of plant cell walls and cell wall components. *Methods Enzymol.* **118**:3-40 (1985).

132. Vidal, S. *et al.*, Structural characterization of the pectic polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain, *Carbohydr. Res.* **326**:277-294 (2000).

133. Whitcombe, A.J. *et al.*, Structural characterization of the pectic polysacchride, Rhamnogalacturonan-II, *Carbohydr. Res.* **271**:15-29 (1995).

134. Boyes, D.C. *et al.*, Growth stage-based phenotypic analysis of arabidopsis: a model for high throughput functional genomics in plants, *Plant Cell* **13**:1499-1510 (2001).

135. Zablackis, E. *et al.*, Characterization of the cell-wall polysaccharides of *Arabidopsis thaliana* leaves, *Plant Physiol.* **107**:1129-1138 (1995).

136. Madson, M. *et al.*, The MUR3 gene of Arabidopsis encodes a xyloglucan galactosyltransferase that is evolutionarily related to animal exostosins, *Plant Cell* **15**:1662-1670 (2003).